Award Number: W81XWH-11-1-0766

TITLE: Detection of Xenotropic Murine Leukemia Virus Related Virus (XMRV) in Gulf War Illness: Role in Pathogenesis or Biomarker?

PRINCIPAL INVESTIGATOR:
Vincent C. Lombardi

CONTRACTING ORGANIZATION:
Whittemore Peterson Institute
Reno, NV 89557

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Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of this project is to evaluate subjects with Gulf War illness (GWI) for pathogens and potential biomarkers. The scope of this work will primarily encompass transcriptional analysis of lymphocytes from individual with GWI and compare the results to the same data from healthy control volunteers. Any potential pathogens identified will be confirmed by complimentary methods. Additionally, we will also survey the production of inflammatory cytokines and chemokines from the same individuals as well as evaluate lymphocyte populations. This information will be used to produce a diagnostic signature that can be used to delineate those who suffer from GWI from those who do not. At this time, we are developing a potential diagnostic signature that can be used to distinguish GWI from healthy controls and a closely related illness, chronic fatigue syndrome (ME/CFS).

15. SUBJECT TERMS
GWI, cytokines, next generation sequencing

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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INTRODUCTION:

The aim of this project is to evaluate subjects with Gulf War illness (GWI) for pathogens and potential biomarkers. In order to fulfill this mandate, we will focus on evaluating the transcriptome of circulating lymphocytes as well as any potential blood-associated pathogens. The transcriptome is reflective of the genes that are being actively expressed at any given time; therefore, the lymphocyte transcriptome represents a window into the immune system, potentially leading to an understanding of GWI pathogenesis. Transcriptome analysis also has the ability to identify any pathogens present in the immune cells or circulating in the blood, thus, potentially identifying an etiological trigger. We will also evaluate inflammatory cytokines and chemokines and use this data to develop a diagnostic signature. By identifying potential biomarkers including pathogens associated with GWI, the results of this study may afford physicians the necessary tools to make more accurate diagnoses and improve subject care. In order to conduct these aims, we will recruit up to 100 subjects/veterans who were on active duty during the Gulf War era (Desert Storm: 1990-1991) and have symptoms of GWI as well as 100 subjects who have no symptoms of GWI.

BODY:

The original proposed aim of this study was to establish the prevalence of a newly identified infectious retrovirus in individuals with GWI. The original Principal Investigator (PI) left the Whittemore Peterson Institute ("WPI") shortly after this study was funded. Therefore, a petition was made to have the PI changed to Dr. Vincent Lombardi. In light of several subsequent research reports that questioned the possibility of this newly identified retrovirus as a human pathogen, Dr. Lombardi also made a request to the Army Contracting Officer Representative to amend the original proposal to broaden the scope of the pathogen discovery aspect of this study by utilizing next generation sequencing (NGS) technology to allow any pathogen to be identified, including, but not limited to, the originally proposed retrovirus. Additionally, this technology has the potential to identify useful biomarkers and immune dysregulation through transcriptome analysis, which was not addressed in the original proposal, but was incorporated into the amended proposal.

Upon receiving approval to implement the requested modifications to the original proposal, a new human subjects protocol was required. The WPI and the VA Sierra Nevada Health Care System ("VASNHCS") worked together to define the study population and prepare and submit the protocol, including all consent forms and recruitment materials. The subject protocol was approved by the University of Nevada Reno (UNR) Institutional Review Board (IRB) on June 26, 2012, and by the VA Sierra Nevada Health Care System (VASNHCS) Research and Development Department (R&D) on July, 26 2012. The subject protocol was also reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, U.S. Army, and USAMRMC human subjects protection requirement on August 1, 2012. Additionally, a modification to the IRB allowing for the use of new recruiting documents was approved on September 25, 2012. This completes Task 1a as outlined in the Statement of Work (SOW) for months 0-6 as predicted in the proposed timeline.

After approval of the protocol, the VASNHCS commenced recruiting operations. This process is ongoing, and we have just concluded our twelfth month of subject recruitment. We anticipated recruiting 50 GWI cases and 50 healthy control subjects as of this date; however, at this time, we have recruited 67 total subjects (41 cases and 26 controls). The recruitment is less than we had anticipated so we are slightly delayed in commencing our NGS analysis. Our initial SOW indicated that we would begin NGS analysis after 30 subjects from each group are recruited. Therefore, we are near reaching this limit and analyses will commence once three more control subjects have been recruited (see reportable outcome section). Study subjects with GWI continue to be recruited through

direct contact and both GWI and healthy control subjects are being recruited through advertising flyers posted at the VASNHCS facilities, UNR campus, and local veterans organizations (Task 1b of the SOW, 1-18 months predicted in the study timeline). A critical component of this research project was the acquisition of the NGS platforms. As of this date, two NGS platforms have been purchased through a joint collaborative effort between the WPI and UNR. These instruments (the Life Technologies Personal Genome Machine and the Life Technologies Ion Proton) are now housed at UNR's genomics core facility, and we are currently utilizing them to generate data for this project.

To maintain productivity during the course of subject recruitment and sample processing, we commenced analysis for other immune parameters including lymphocyte population and enumeration by flow cytometry and inflammatory cytokine and chemokine expression. As a result of our preliminary findings regarding the expression of inflammatory cytokines and chemokines, we also began conducting studies on the kinetics of inflammatory cytokine expression from primary lymphocytes *ex vivo*. We have elected to focus on the activation of toll-like receptors as a primary trigger for cytokine production.

Toll like receptors (TLRs) are pattern recognition receptors that are activated by molecular motifs shared by pathogens, but that are typically not present in the host. There are several well-defined TLRs with specific activating ligands. For example, TLR4 is activated by the bacterial product lipopolysacharide (LPS) while single stranded RNA and bacterial DNA, typically of viral origin; activate TLR 7 and 9, respectively. Activation of various TLRs can trigger the expression of a unique pattern of cytokines, influencing disease progression and outcome. As part of our investigation into the control of inflammatory cytokines, we sought to determine the effect upon stimulation of various TLRs expressed by PBMC and plasmacytoid dendritic cells from GWI cases and control donors. The TLR4 agonist, LPS and TLR7 and 9 agonists Imiquimod (IMQ) and ODN 2216 (ODN), respectively, were used to study the inflammatory kinetics associated with GWI. TNFα was chosen to follow TLR4 stimulation and INFα was used to follow TLR 7 and 9 stimulation.

Prepherial blood mononuclear cells (PBMCs) ($2x10^5$) from GWI cases and controls were stimulated with LPS (10 ng/ml and 1µg/ml) directly after seeding culture plates, followed by stimulation at 16, 24, 32, 38, and 48 hours. Culture medium was harvested at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 26, 32, 34, 38, 40, 48, and 50 hours after LPS stimulation and stored at -80 °C until assayed. Culture medium was replaced each time with fresh medium. PBMC stimulation response is presented in Figure 1. pDCs were isolated using Miltenyi CD304 (BDCA-4/Neuropilin) microbead kit. pDCs were rested for 18 hours after isolation before adding stimulants. pDCs were stimulated with IMQ (5 ug/mL) or ODN (5 uM). IMQ and ODN stimulation response and culture medium harvesting was performed similar to experiments with LPS stimulation of PBMC. Concentration of TNFα and INFα were determined by microplate ELISA.

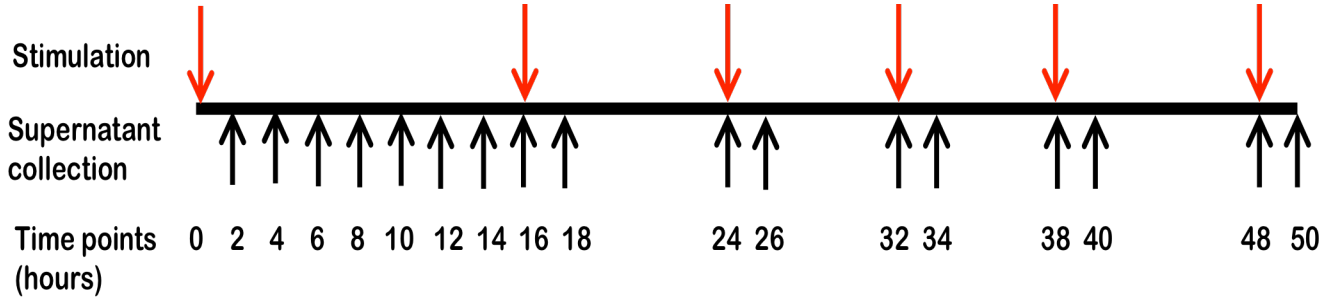

Fig.1 PBMC stimulation experiment; mode of stimulation and supernatant collection.

LPS (10 ng/ml) was added to the PBMC cultures at 0, 16, 24, 32, 38, and 48 hours. Concentration of TNFα in supernatants was determined at selected time points. With exception of 24 hours post

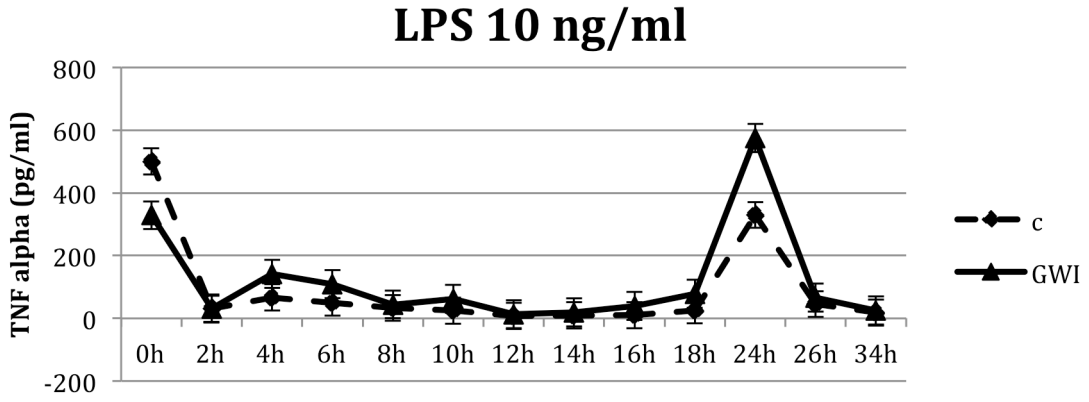

Fig. 2. TNF-α activation in PBMC from GWI subjects and healthy controls stimulated with LPS (10 ng/mL).

stimulation, TNFα concentration did not differ significantly in supernatants of PBMC collected from GWI cases and controls (Fig 2).

At 24 hours, TNFα concentration in the supernatant of PBMC from GWI cases was significantly higher compared to that in controls. Our data suggests that PBMC from GWI subjects maintain ability to activate TNFα when stimulated with low concentration of LPS similar to that in controls but produce slightly more TNFα at 24 hrs.

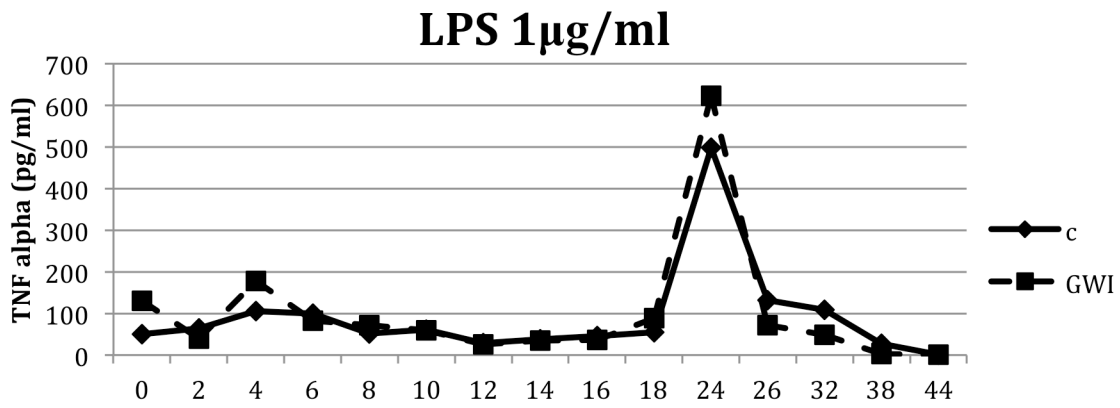

Fig 3. TNFα activation in PBMC from GWI subjects and healthy controls stimulated with LPS (1µg/ml).

Next, we sought to determine the effect of high concentration of LPS (1 µg/ml) on TNFα activation in PBMC from GWI cases and controls. The mode of LPS stimulation and supernatant collection is the same as described in Fig 1. High concentration of LPS (1 µg/ml) produced a similar pattern of TNFα activation as that produced by low concentration of TNFα (10 ng/ml) (Fig 3). Since LPS acts through activation of TLR4, we conclude that PBMC from GWI cases retains ability to respond to TLR4 agonists similar to that of healthy donors at high concentrations, but GWI cases are more sensitive to low level LPS stimulation. These data suggest that GWI cases may respond to bacterial-induced inflammation to a greater extent than control subjects.

Next, we sought to determine the effect of ODN and IMQ on activation of INFα in pDCs from GWI subjects and healthy controls. Cell culture supernatants were collected at selected time points and used to determine INFα concentration in ELISA assay.

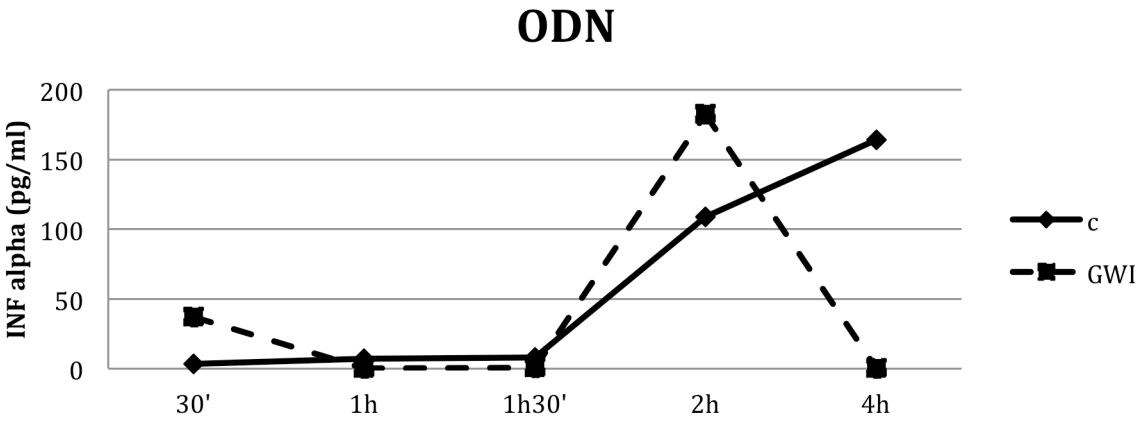

Fig. 4. Effect of ODN stimulation on INFα production by pDCs from GWI subjects and healthy subjects.

INFα concentration in supernatant pDCs from controls was increased at 2 and 4 hours after stimulation with ODN (Fig 4). Levels of INFα in supernatant of ODN stimulated pDCs from GWI cases increased similar to that in controls by 2 hours after stimulation. However, levels of INFα declined, returning to initial levels by 4 hours post stimulation with ODN. IMQ activated INFα in pDCs from controls at 1-hour post stimulation. In contrast, INFα production was observed to be negligible in

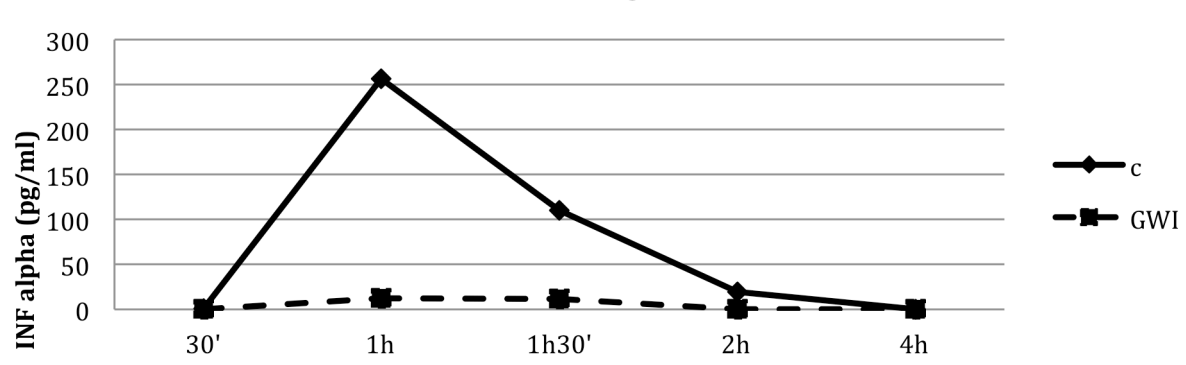

Fig. 5 Effect of IMQ on TNFa activation in pDC from GWI subjects.

supernatant of IMQ stimulated GWI cases (Fig 5). In summary, we conclude that TLR-associated function of pDCs from GWI subjects is abnormal when compared to control subjects. pDCs from GWI subjects show initial TLR 9 activity, but fail to maintain activation as compared to that of controls. Additionally, TLR7 function in pDCs from GWI subjects is significantly suppressed.

Next, we sought to determine cytokine and chemokine concentration in serum of GWI subjects (Fig 6). Cytokine profile of serum from GWI subjects were analyzed initially for 19 GWI cases and 19 controls and revealed decreased concentration of IL8, MIP-1β, MCP1, IL15, IL-1β, and IL-1α. IL8 and MIP-1β beta are produced by pDCs and are involved in regulation and maintenance of pDCs function.

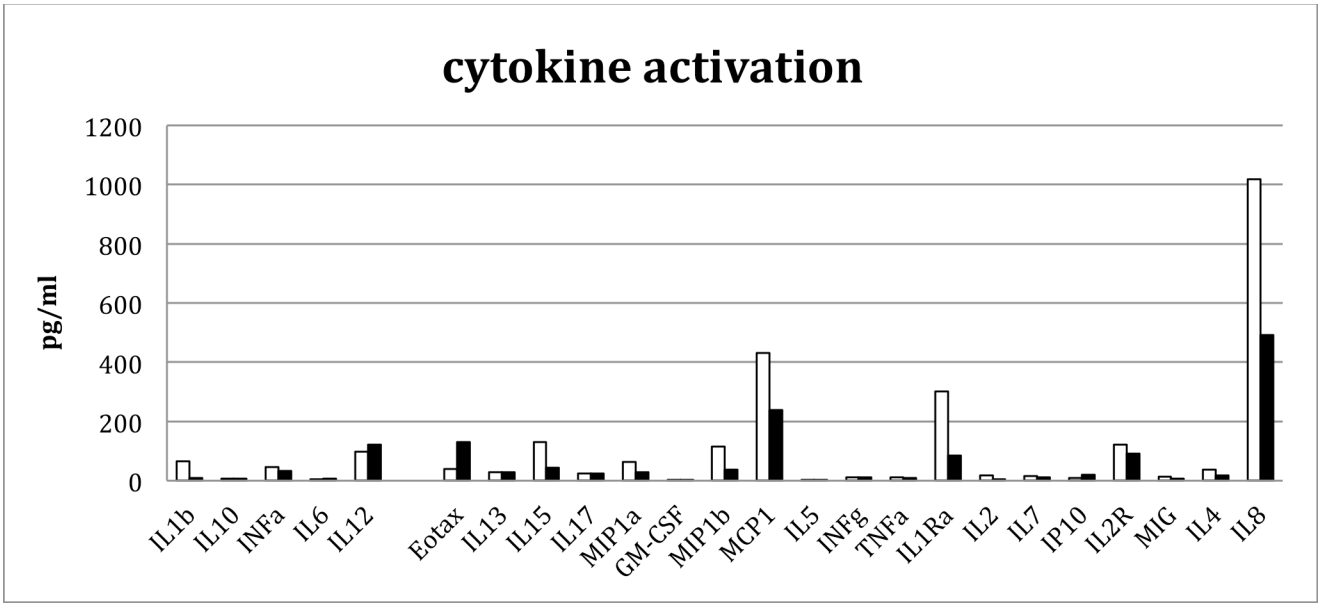

Fig.6. GWI subjects' serum cytokine concentration. White bar is healthy subjects; black bar is GWI subjects.

Presently, we are expanding upon this initial cytokine analysis by screening GWI subjects and healthy controls for 60 serum cytokine and chemokine. We will use this information to potentially develop a diagnostic algorithm. To this end, we have used the Random Forest (RF) classification algorithm [1] to construct a predictive algorithm. The RF algorithm uses an ensemble of unpruned classification or regression trees produced through bootstrap sampling of the training data set and random feature selection in tree generation. Prediction is made by a majority vote of the predictions of the ensemble. The strength of the analysis was evaluated by an "out of bag" sampling without replacement of the original data. The RF is an attractive method since it handles both discrete and continuous data, it accommodates and compensates for missing data, and it is invariant to monotonic transformations of the input variables. The RF algorithm is uniquely suited for cytokine and chemokine analysis in that it can handle highly skewed values well and weighs the contribution of each cytokine or chemokine according to its relatedness with others. To develop a useful diagnostic algorithm, it is necessary to discriminate cases from controls, but also to discriminate cases from diseases with similar or over-lapping symptoms. Using cytokine and chemokine values as the predictor variable and subject diagnosis (GWI subjects, ME/CFS subjects and healthy controls) as the target variable, we have preliminarily produced a diagnostic algorithm that can be used to identify ME/CFS cases with 90.77% sensitivity and GWI cases with 78.9% sensitivity (Table 1). While the specificity of the model remains low, it should improve with additional data upon further study recruitment. Overall, the model is only 69.59% accurate; however, we believe that once we have additional data from the NGS results, we can refine this model to produce the specificity and sensitivity necessary for a clinical diagnostic.

Table 1

Prediction success

Actual Class	Total Class	Percent correct	Con N = 28	GWI N = 53	CFS N = 67
Control	45	31.11%	14	23	8
GWI	38	78.95%	8	30	0
ME/CFS	65	90.77%	6	0	59
Total:	148				
Average:	Total Class	66.94%			
Overall % Correct:		69.59%			

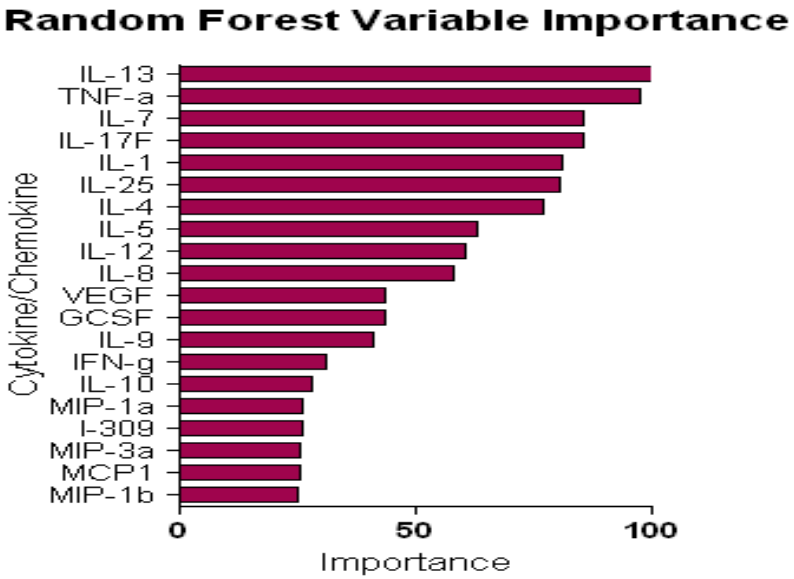

Figure 7. Random Forest prediction. Horizontal bars represent the relative importance that each cytokine or chemokine contributes to the predictive nature of the signature. Only the most significant 20 cytokines are shown. These data also suggest the most important cytokines that contribute to the pathogenesis of the disease and also suggest lymphocyte populations involved in pathogenesis.

Our preliminary NGS analysis suggests that using individual lymphocyte populations will provide more useful data in contrast to transcriptome analysis of whole blood. As part of our cytokine kinetics analysis and our RF model, we have potentially identified the most significant cytokines and chemokines that can be used to delineate cases from controls (Figure 7) and used this knowledge to choose the best possible candidates for cell sorting [2-5]. For instance, we have determined that pDCs may be abnormal in GWI cases; however, in that pDCs only represent approximately 0.1% of total lymphocytes [6], their transcripts could easily be lost in a background of total lymphocytes. Therefore, based upon our analysis, we have chosen to sort five populations of lymphocytes (monocytes, CD4 T cells, CD8 T cells, CD4/CD8 double neg T cells, and pDCs; Figure 8).

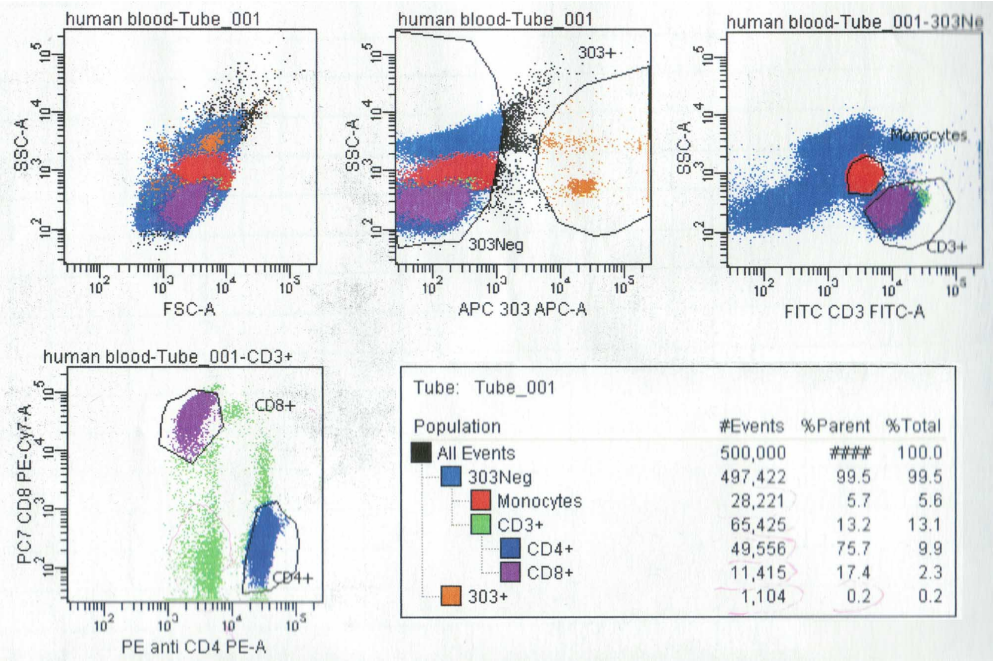

Figure 2. Sorting scheme for purifying lymphocyte populations for RNAseq. Plasmacytoid dendritic cells are sorted as CD303 positive. CD303 negative cells are sorted into monocytes as CD91 positive and T cells as CD3 positive based on granularity. CD3 cells are further sorted into CD4 positive, CD8 positive cells. Once a significant number of CD4/CD8 cells are collected, CD4/CD8 double negative cells are collected.

KEY RESEARCH ACCOMPLISHMENTS:

Because of the change in the scope of work and resulting requirement for new protocol approvals, the ability to recruit and consent study subjects was delayed. Consequently, key research accomplishments, relating to the NGS protion of this study, can only be realized once a sufficient number of study subjects are recruited, allowing for the first batch of NGS sequencing and subsequent data analysis to be completed.

1. **Identification of a dysregulation in the type I interferon response in GWI to TLR7 activation.**
2. **Identification of an increased response to TLR4 activation in GWI.**
3. **Identification of cytokines and chemokines that differentiate GWI from a closely related disease.**

REPORTABLE OUTCOMES:

The analysis of our flow cytometery data is ongoing; however, our preliminary analysis regarding the identification of CD56Neg NK cells has contributed to the generation of the manuscript title “Properties of Human Lymphocytes Expressing Perforin (PRF1) I.: Natural Killer (NK) Cells Defined as CD3NegPRF1+ Lymphocytes Include CD56Neg Cells in Healthy Subjects”. This manuscript was submitted to the Journal Cytometry B and has been accepted with revisions.

CONCLUSION:

The original proposed aim of this study was to establish the prevalence of a newly identified infectious retrovirus in individuals with GWI; however, several subsequent research reports brought into question the likelihood of its involvement. Therefore, in order to provide the greatest possibility of success, the proposed methods of the original proposal were modified to incorporate transcriptome analysis using NGS. This modification provides for methods that have the ability to identify any potential pathogen involved in GWI pathogenesis, thus, preserving the original mandate. The proposed methods of transcriptome analysis also allow for potential biomarkers to be identified. Although the change in PI, the modification to the study design, and the necessity for new human subject protection applications has resulted in a significant delay, study subject recruitment and

enrollment is proceeding. We have concluded that transcriptome analysis of whole blood is not likely to provide the best results when developing a diagnostic algorithm, however, we have used our preliminary data to choose the best candidates for cell sorting and transcriptome analysis of individual cell populations. This work is currently underway. Once we have completed sorting and sample preparation of the final study subjects we will commence the bioinformatics phase of this study.

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APPENDICES:

1. Revised Statement of Work
2. Change in IRB Protocol
3. IRB Protocol
4. IRB Protocol approval
5. VA R&D Approval
6. HIPAA Waiver Approval
7. DOD Final Approval
8. Invitation to Participate Letter
9. Follow up Phone Script
10. VA Research Consent Form
11. IRB Modification Request Form
12. IRB Approval for Recruitment Materials
13. Participant Questionnaire
14. Recruitment Flyer Case Subject
15. Recruitment Flyer Control Subject

SUPPORTING DATA:

None

W81XWH-11-1-0766
Statement of Work Revised 1/11/12

Pathogen and Biomarker Discovery in Gulf War Illness
Vincent C. Lombardi, Principal Investigator

Statement of Work: The overall goal of this project is to identify potential pathogenic agents and immune markers in a cohort of patients with Gulf War illness ("GWI"). Next generation transcriptome sequencing will be utilized to compare all actively expressed genes in GWI patients, to that of an unrelated healthy control population. This information will be used to identify significant differences in immune parameters and any underlying pathogens potentially contributing to the origination and development of GWI.

Study Performance Site 1: Whittemore Peterson Institute ("WPI")
University of Nevada, Reno MS 0552
1664. N Virginia St.
Reno, NV 89557

Study Performance Site 2: VA Sierra Nevada Health Care System ("VASNHCS")
Ioannis A. Lougaris VA Medical Center
1000 Locust St.
Reno, NV 89502

Study Performance Site 3: University of Nevada, Reno ("UNR")
1664. N Virginia St.
Reno, NV 89557

Tasks 1-3 accomplish Aim 1 of the proposal.

Task 1:
A total of 50 GWI patients and 50 controls will be accrued each year of the two-year study period. The control subjects recruited will be healthy individuals, not necessarily related to GWI patients or healthy military personnel as originally proposed. VASNHCS will be principally responsible for the recruitment and consenting of research subjects at Study Performance Site 2 and the VA Sierra Nevada Health Care System Community Based Outpatient Clinics ("CBOCs") as appropriate. The VASNHCS budget includes a study coordinator for these efforts.

1a) 0-6 months following award notice-funding: WPI and VASNHCS will work together to define the study population and prepare and submit the IRB application for the project, including all consent forms and study advertising such that study is approved as soon as possible following award notification.

1b) 1-18 months following award notice-funding: VASNHCS will recruit and consent research subjects.

Task 2: VASNHCS will draw blood samples (1-8ml lavender cap tube, 1-8 ml green cap tube, 1 8-ml serum separator tube and 1-2.5 ml PAXgene blood RNA tube) from research subjects at

Study Performance Site 2 and CBOCs as appropriate and ship or carry to WPI at Study Performance Site 1 within 48 hours of draw (months 1-21). The VASNHCS budget includes costs for blood draws.

Task 3: In order to initially establish differences in immune parameters and identify potential pathogens, WPI will perform lymphocyte transcriptome analysis of 30 GWI subjects and 30 healthy controls, using next generation sequencing technology (see note 1). A complete analysis will subsequently be made of the entire subject population by RT-PCR using the genes and pathogens identified by the transcriptome analysis. The WPI budget includes costs for research personnel and materials for the testing and analysis described in Tasks 3 and 4.

Study Performance Site 2 (months 1-21).

3a) RNA extraction and mRNA truseq library preparation

3b) Next generation sequencing (Illumina HiSeq 1000) at 50bp read length

3c) Post sequence assembly

3d) Transcript identification through bioinformatic analysis of data

Tasks 4 and 5 accomplish Aim 2 of the grant proposal.

Task 4. WPI will clone and characterize any novel pathogens identified and perform transcript confirmatory analysis of immune markers by reverse transcriptase PCR and protein based detection methods at Study Performance Site 1 (months 6-24).

Task 5. Statistical analysis to determine differences between patient and control groups will be performed by UNR personnel at Performance Site 3. Dr. Julie Smith Gagen will propose methods to analyze de-identified data and write computer programs to analyze data. Under the direction of Dr. Smith-Gagen, Dihalia Fuentes will provide data management, run computer programs, create tables and present results (months 4-24). The UNR budget includes personnel costs only.

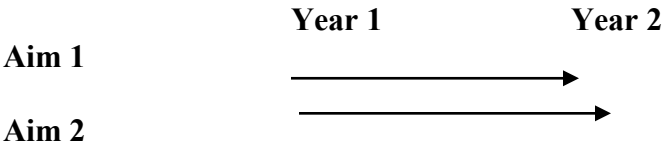
Note 1. Previous reports suggest the up regulation of a set of 10 cytokines and chemokine in patients with similar symptoms (Lombardi et al. In Vivo 2011). Using this preliminary data, we calculated the power of our study to identify the up regulation of these cytokines. Given that 96% of cases and 92% of controls were accurately differentiated using a Random Forrest generated cytokine profile in the previous study, we need a minimum of 12 cases and 12 controls to obtain a significance level of 0.01 and 99% power to detect differences between cases and controls based on a RF generated cytokine profile. We have increased this value to 30 patients and 30 controls to compensate for potential differences.

Lombardi et al, In Vivo May-June 2011 vol. 25 no. 3 307-314

Casagrande JT, Pike MC, Smith PG. An improved approximate formula for calculating sample sizes for comparing two binomial distributions. Biometrics 1978;34:483-486.

Fleiss JL. Statistical Methods for Rates and Proportions (2nd edition). New York: Wiley 1981

Timeline
The timetable for the experiments proposed in this proposal is indicated below.



University of Nevada, Reno

Office of Human Research Protection
205 Ross Hall / 331, Reno, Nevada 89557
775.327.2368 / 775.327.2369 fax
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Protocol Modification Form

Protocol Number: B12-007 **Date:** 12 October 2011
Principal Investigator: Vincent Lombardi, PhD
Protocol Title: Detection of xenotropic murine leukemia virus-related virus (XMRV) in Gulf War Illness: role in pathogenesis or biomarker?

1. Proposed Changes:

☒ **Protocol Modification or Amendment:**
- ✓ Required: Attach 2 copies of revised protocol application form with updated version date.
- ✓ If applicable: Attach 2 copies revised supporting document(s).
- ✓ If applicable: Attach 2 copies revised consent document(s) with updated version date.

Brief description of modifications:

Replace Dr Judy Mikovits with Dr Vincent Lombardi as Principal Investigator. Dr. Lombardi will now be the Principal Investigator and Dr. Mikovits will no longer be involved with the research.

Reason for modifications:

Dr Mikovits is no longer with Whittemore Peterson Institute. The granting organization (DoD Congressionally Directed Medical Research Program [Gulf War Illness Research Program]) is aware of this change and is revising their records to reflect Dr Lombardi as the principal investigator for the grant.

Dr Davis' phone number in consents and phone script is changed to 775-328-1464.

☒ **Revised Consent Documents:**
- ✓ Required: Attach 2 copies of revised consent document(s) with updated version date.

2. New Principal Investigator (PI):
- ✓ Required: Attach 2 copies of revised protocol application form with updated version date, **signed by the new PI**.
- ✓ If applicable: Attach 2 copies of revised consent document(s) with updated version date to reflect PI change.

Current PI: Dr Judy Mikovits

New PI: Dr Vincent Lombardi

3. Co-investigator(s) / Research Personnel:
- ✓ Required: Attach 2 copies of revised protocol application form with updated version date.
- ✓ If applicable: Attach 2 copies of revised consent document(s) with updated version date to reflect personnel changes.

☒ Add co-investigator / research personnel name(s): Svetlana Khaiboullina

☐ Remove co-investigator / research personnel name(s): _____

4. Change of Sponsor(s):
- ✓ Required: Attach 2 copies of revised protocol application form with updated version date.
- ✓ Required: For each new sponsor, attach 2 copies of grant proposal and/or contract with scope of work.
- ✓ If applicable: Attach 2 copies of revised consent document(s) with updated version date.

☐ Add Sponsor(s) name: _____

☐ Remove Sponsor(s) name: _____

5. Principal Investigator Signature:

Assurance: I hereby certify that all information provide with this request is complete and accurate. For investigator/personnel changes: I hereby certify that all responsible investigators added above are appropriately credentialed and/or trained to perform their role in this protocol. I further certify that the participation of any co-investigators or research personnel listed above does/do not, in any way, violate the University of Nevada, Reno policy on conflicts of interest.

_____

Signature of Principal Investigator (or **Responsible Official) Date

(**PI Changes only: Current PI must sign, if submitting the change, or Responsible Official must sign, if current PI unavailable)

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Protocol Application for the Involvement of Human Subjects

Version Date: 05/30/12 UNR Protocol #: B12-036
(*Version Date required; may not be handwritten*)

SECTION I: General Information

1. Submission Type [Submit two complete copies]

☐ Full Board (the research poses greater than minimal risk to the subjects)

☒ Expedited Review (minimal risk research) → **Complete and Attach Expedited Review Checklist**

2. Research Type

☒ Biomedical ☐ Social Behavioral

3. Study Title: Pathogen and Biomarker Discovery in Gulf War Illness

4. Abstract: In 250 words or less, provide a brief abstract of the proposed research in language that can be understood by a non-scientist. Summarize the background, study purpose, study method and procedures, and the anticipated research findings.

Gulf War illness (GWI) is a chronic multi-symptom disorder affecting approximately one third of the veterans and civilians who served in Persian Gulf War (Desert Storm 1990-1991). It is a syndrome primarily described by a spectrum of symptoms, innate immune abnormalities and opportunistic infections. A wide range of acute and chronic physical symptoms are associated with GWI including fatigue, musculoskeletal pain, gastrointestinal dysfunction and cognitive problems. Unfortunately, diagnosis of GWI is difficult in that no discrete biomarkers or etiological agents have been identified. A greater understanding of the innate immune system and the associated opportunistic pathogens may provide insight into the pathophysiology of this disease. In order to explore this issue, we will conduct lymphocyte transcriptome analysis of subjects diagnosed with GWI and compare the results to that of healthy control subjects. The transcriptome is reflective of the genes that are being actively expressed at any given time; therefore, the lymphocyte transcriptome represents a window into the innate immune system, potentially leading to an understanding of GWI pathogenesis. Transcriptome analysis also has the ability to identify any pathogens present in the immune cells, potentially identifying an etiological trigger. The goal of this study is to identify potential biomarkers, including pathogens, associated with GWI, which in turn will afford physicians the necessary tools to make more accurate diagnoses.

5. Type of Study

(Check all that apply)

☐ Faculty Research
☒ VA Research
☐ Student Research
- ☐ Undergraduate Honors Thesis
- ☐ Comprehensive Project
- ☐ Thesis (Must be approved by the Student Investigator's thesis committee prior to submission)
- ☐ Dissertation (Must be approved by the Student Investigator's dissertation committee prior to submission)
- ☐ Other → specify:

☒ Other → specify: Whittemore Peterson Institute for Neuro-Immune Disease (WPI)

6. Principal Investigator (Only one investigator may serve as the PI.)
Note: Students may be PI only on applications for exempt research.

Name and Degree(s): Vincent Lombardi, PhD
Mailing Address: 1664 N Virginia St, University of Nevada, Reno, 89557, MS 0552
Department: WPI
Email: vclombardi@wpinstitute.org Phone: 775-682-8278 Fax: 775-682-8258

7. Student Investigator
(Complete only for student-initiated research; students working on faculty-initiated research should be listed in item 9 below. All student research must have a faculty member as the Principal Investigator.)

Name and Degree(s): _____
Mailing Address: _____
Department: _____
Email: _____ Phone: _____ Fax: _____

8. Contact Person
(You may identify an investigator, student, or staff member to serve as the primary point of contact for all correspondence.)

☐ Check here if same as Student Investigator

Name and Degree(s) Vincent Lombardi, PhD
Mailing Address: 1664 N Virginia St, University of Nevada, Reno, 89557, MS 0552
Department: WPI
Email: vclombardi@wpinstitute.org Phone: 775-682-8278 Fax: 775-682-8258

9. Study Personnel and Roles
List all research personnel associated with this project. Attach training documentation for personnel with training other than UNR or VA.

Name and Degrees(s)	**Title on Project** Principal Investigator = PI (only one person may serve as PI); Co-Investigator = Co-I; Research Team Member = RTM		**Training Verification** Check one: UNR	VA	Other (attach)	**Actual Role on Project** Specify the responsibilities of each individual listed, e.g. study coordination; obtain consent: recruitment; assessments; data collection; data analysis; etc.
John Researcher, Ph.D.	PI		☐	☐	☐	e.g. Responsible for obtaining consent; data collection
Vincent Lombardi, Ph.D.	PI		☒	☐	☐	Responsible for the direction of research, sample sequencing and data analysis
Svetlana Khaiboullina, M.D., Ph.D.	☐ Co-I	☒ RTM	☒	☐	☐	Responsible for sample preparation for pathogen identification; quantitative PCR method development; KIR analysis; and development of experimental models using previously developed cell lines
Shanti Rawat, M.S.	☐ Co-I	☒ RTM	☒	☐	☐	Responsible for all initial sample processing and cell culture work; assist with sample preparations such as DNA extraction and cDNA preparations
Rory Berk	☐ Co-I	☒ RTM	☐	☒	☐	Responsible for recruitment and obtaining consents
Julie Smith Gagen, Ph.D.	☐ Co-I	☒ RTM	☒	☐	☐	Responsible for data analysis
Sheila Young, Ph.D.	☒ Co-I	☐ RTM	☐	☒	☐	Responsible for recruitment
Elizabeth Hill, Ph.D.	☒ Co-I	☐ RTM	☐	☒	☐	Responsible for project direction, recruitment and obtaining consents
_____	☒ Co-I	☐ RTM	☐	☐	☐	_____
_____	☐ Co-I	☐ RTM	☐	☐	☐	_____
_____	☐ Co-I	☐ RTM	☐	☐	☐	_
_____	☐ Co-I	☐ RTM	☐	☐	☐	_____

10. Research Responsibilities
The Principal Investigator will ensure that all study personnel are adequately informed about the protocol and their research-related duties with:

☒ Routine Meetings ☒ Regular Communication (e.g. email, phone conferences, etc.) ☐ Other → specify: _____

11. Performance Sites

11.a. Study Locations (Check all that apply) **(NOTE: Permission letters are required from all non-UNR sites)**

☒ UNR Campus ("Campus" includes main campus, UNSOM, UNCE, Warren Nelson Building, Redfield Campus, CASAT, Sanford Center for Aging)

- ☐ Classroom
- ☐ Clinic
- ☐ Lab
- ☐ Other→ specify: _____

Affiliates:

☒ VA Sierra Nevada Health Care System (VASNHCS). **Required:** Attach review from VASNHCS Protocol Review Subcommittee.

- ☐ Desert Research Institute (DRI)
- ☐ Truckee Meadows Community College (TMCC).
- ☒ Whittemore Peterson Institute (WPI)

Affiliates with reciprocal IRB agreements (contact the OHRP at (775) 327-2368 about IRB requirements):

- ☐ University of Nevada, Las Vegas (UNLV).
- ☐ Renown Regional Medical Center.
- ☐ St. Mary's Regional Medical Center (a member of Catholic Healthcare West)

Non-Affiliates:

- ☐ Off campus, non-affiliated performance site named here:
- ☐ Washoe County School District. **Required: Letter of permission to use WCSD site.**
- ☐ Web-based research

11.b. Describe how the facility or site in which the research will be conducted is appropriate for the project and protects the participants' privacy.

The informed consent process, completion of participant questionnaire and blood draw will be conducted in private rooms at the Ioannis A. Lougaris VA Medical Center (Reno) or the nearest VASNHCS Community Based Outpatient Clinics (CBOCs). These rooms have doors that seclude paticipants from all others, maintaining their privacy and confidentiality. The process and analysis of samples will be conducted in the laboratories at the Whittemore Peterson Institute (WPI) in the Center for Molecular Medicine (CMM), Building 160, at the University of Nevada, Reno (UNR): a 1250 sq. ft. basic research laboratory housed in Room L-300 of the CMM and a 2,480 sq. ft. clinical laboratory housed in room 315 of the CMM East Wing.

Both laboratories contain the required equipment to complete the study goals and will be used interchangably for sample culture, protein expression, DNA extraction, sequencing, cDNA preparations, quatitive PCR, pathogen identification, KIR analysis and the development of experimental models using previously developed cell lines. The basic research laboratory houses a dedicated PCR room with two dead air boxes, an Eppendorf thermal cycler, a Bio-Rad gradient thermal cycler, a Bio-Rad Gel Doc EZ imager, a Perkin Elmer Victor X3 multilable plate reader and a Cepheid Smart Cycler. It also has a dedicated cell-culture room, two Class IIB tissue culture hoods, an Eppendorf 5415R microcentrifuge, an Allegra X-15R tabletop centrifuge and a Zeiss Observer A.1 inverted fluorescent microscope. The clinical laboratory has extensive cell-culture facilities including a dedicated cell-culture room, three Class IIB tissue culture hoods, three CO2 tissue culture incubators, an Olympus inverted phase contrast microscope, an AMG EVOS Digital Inverted Fluorescent Microscope, a Sorvall refrigerated microcentrifuge, a Sorvall Legend refrigerated tabletop centrifuge and a Perkin Elmer Victor X5 multilable plate reader. In addition, it houses two PCR rooms (one for single round amplification and one for second round nested PCR amplification) with two dead air boxes, two thermal-cyclers (a Bio-Rad CFX96 Real-Time PCR Detection System and an Eppendorf gradient thermal-cycler), and a Bio-Rad Chemidoc imaging system. In addition, the University of Reno is in the final stages of purchasing an illumina HiSeq 1000 Next Generation Sequencing system that will also be used in this research study.

To protect the identity of subjects, samples will be sent to the laboratory coded (de-identified) and they will be stored in locked -70 deg. freezers and 2 LN2 (liquid nitrogen) tanks in the WPI research lab (Room L-300) at all times unless they are being processed by the PI or designated research team member. All research team members have completed the required CITI and VA training and they will follow developed Standard Operational Procedures (SOPs) that reinforce the IRB's mandates and protect participants' privacy and confidentiality.

11.c. ☐ This study has been/will be reviewed by another IRB.

Name of collaborating institution: ____

Name and contact information for the other IRB(s): _____

Describe the procedures for dissemination of protocol information (IRB initial and continuing approvals, relevant reports of unanticipated problems, protocol modifications, and interim reports) between all participating organizations: _____

Attach a copy of the IRB decision and approved consent documents.

SECTION II: Funding

12. Funding Status

☒ This project is funded. **(NOTE: Attach a complete copy of the Grant(s)/Contract(s) supporting this project in whole or in part.)**

Funding Source	Name of Department, Agency, Sponsor, or Source
☒ Federal	Department of Defense Gulf War Illness Research Program of the Office of Congressionally Directed Medical Research Programs
☐ State of Nevada	_____
☐ Local Government	_____
☐ Industry For-Profit	_____
☐ Private / Non-Profit	_____
☐ Internal (UNR/UNSOM)	_____
☐ Personal Funds	_____
☐ Other	_____

Principal Investigator of Grant/Contract: Vincent Lombardi
Grant/Contract Title: Pathogen and Biomarker Discovery in Gulf War Illness
Grant Number: W81XWH-11-1-0766
Grant/Contract Status (e.g., pending, awarded): awarded

13. Conflict of Interest

For externally funded studies:

☐ The PI or co-investigator(s), or their families, or any other member of the research team, or their families, has a Significant Financial Interest (SFI), defined as a value that exceeds $10,000, related to the proposed research.

Please provide the following information for all investigators and members of the research team with a significant financial interest (SFI).

Name	Has a SFI Disclosure Form been submitted to the Office of Sponsored Projects?	Does the Office of Human Research Protection have a copy of the Management Plan?
_____	☐ Yes ☐ No	☐ Yes ☐ No
_____	☐ Yes ☐ No	☐ Yes ☐ No
_____	☐ Yes ☐ No	☐ Yes ☐ No
_____	☐ Yes ☐ No	☐ Yes ☐ No
_____	☐ Yes ☐ No	☐ Yes ☐ No
_____	☐ Yes ☐ No	☐ Yes ☐ No

Attach a copy of the SFI Management Plan for each individual listed above.

SECTION III: Subjects

14. Total enrollment: 200

15. Subject Population(s) targeted for this study:

Check all that apply:

☒ Adult Volunteers (18 years of age or older)

☐ Children → **Submit Form D: Research with Children**

☐ Prisoners → **Submit Form C: Research with Prisoners**

☐ Pregnant Women, Fetuses, or Neonates → **Submit Form B: Research with Pregnant Women, Human Fetuses and Neonates**

☐ Adults with Impaired Decision-Making Capacity → **Submit Form A: Research with Adults who have Impaired Decision-Making Capacity**

☐ UNR Students (if adults, also check Adult Volunteers above; if any will be under 18 years of age, also check Children above.)

☐ Employees (of the investigators or any other members of the research team)

☐ Economically Disadvantaged

☐ Low Literacy/ Educationally Disadvantaged

☐ Persons whose First Language is not English

☐ Other → specify: _____

16. Inclusion/Exclusion Criteria

List the inclusion and exclusion criteria. Justify all exclusions based on gender (women of childbearing potential), age, or race.

16.a. What characteristics (inclusion criteria) must subjects have to be in this study? Specify for each subject group, if more than one group will be involved.

(a) 100 subjects (veteran GWI patients), male or female, who were on active duty during the Persian Gulf War (Desert Storm: 1990-1991), whether or not actually deployed to Iraq or surrounding areas, and have symptoms of GWI. Subjects primary language must be English.

(b) 100 subjects (control group), male or female, 18 years old or older, who may be a veteran or a non-veteran and have no symptoms of GWI. Subjects primary language must be English.

16.b. What characteristics (exclusion criteria) will exclude subjects from this study? Specify for each subject group, if more than one group will be involved.

(a) GWI subjects will be excluded from this study if subjects were not on active duty during the Persian Gulf War (Desert Storm: 1990-1991) and do not have symptoms of GWI. Also, subjects who have or had a traumatic brain injury (TBI) or have human immunodeficiency virus (HIV) will not be able to participate in the study.

(b) Control group subjects will be excluded from this study if subjects have a diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), traumatic brain injury (TBI), or human immunodeficiency virus (HIV).

For both subjects groups (a and b): Subjects will be excluded from study participation if they self-report being pregnant (hormones in pregnant woman can confound immunological results) and/or have used the following drugs within the last 2 weeks: oral, intravenous, intramuscular, nasal or inhaled corticosteroids; cytokines; methotrexate; or immunosuppressive or cytotoxic agents.

17. Potential for Undue Influence of Subjects

☐ One or more of the listed investigators have relationships with potential subjects that could be construed as a conflict of interest or have the potential to pose undue influence. (Examples: a physician recruiting his/her patients, a professor recruiting his/her students, an employer recruiting his/her employees, and a therapist recruiting his/her own clients as research subjects.)

Describe the relationship(s) and the safeguards that will be in place to minimize the possibility of conflict of interest and undue influence in recruiting subjects and conducting the proposed research. _____

If subjects with Low Literacy/Educationally Disadvantaged will be targeted for this research, please describe the procedures to be used to minimize undue influence in a separate paragraph: ____

SECTION IV: Recruitment

18. Recruitment Procedures:
Investigators must keep in mind that contact with prospective subjects should not significantly intrude upon their privacy.
18.a. Indicate who will recruit subjects.

☐ PI
☒ Co-I(s)
☒ Research Team Member(s)
☐ Other → specify:

18.b. Describe in detail where, when, and how recruitment will take place (i.e. under what circumstances).

Dr. Young, Dr. Hill, and the study coordinator, Rory Berk, will be responsible for recruiting subjects. Subjects will be recruited at the Ioannis A. Lougaris VA Medical Center in Reno and the VA Sierra Nevada Health Care System (VASNHCS) Community Based Outpatient Clinics (CBOCs).

Potential GWI subjects (group a) will be contacted and recruited using a list of screened subjects generated from medical records in the VASNHCS database. Dr. Hill will access the VASNHCS database to screen for eligible GWI subjects (group a) based on diagnostic ICD9 codes which define characteristic symptoms of GWI (ICD9: 780.79, ICD9: 729.1 and ICD9: 780.71 -- See list A in Procedures, Section VI, #30). Subjects will be excluded if their medical records indicate they have or had traumatic brain injury (TBI) or have human immunodeficiency virus (HIV). The contact list of eligible subjects will only include subject's name, phone number and address. A letter will be sent to the potential subject describing the study and providing a point of contact for the investigator and the study coordinator. Unless the potential subject states they do not want to be contacted, they will receive an initial phone call. Phone calls will be made to potential subjects by any of the three recruiters using the list generated from the database. In that first phone call, subjects will have the study described to them and will be provided with contact information in case they have future questions. Recruiters will make a maximum of three attempt calls to contact the potential subject, leaving study coordinator contact information if subject is not there at the moment. After three attempts, one voice mail message will be left (if available). However, the voice mail message will only ask that the call be returned. Information very specific to the study will not be left as a voice mail message. The phone script, letter, and follow up phone script are attached.

Potential healthy control subjects (group b) will be recruited through flyers posted in the Ioannis A. Lougaris VA Medical Center in Reno, the VASNHCS CBOCs, local veterans' service organizations who agree to allow flyers to be posted in their areas, and on the WPI website. Tthe flyer is attached. If interested in participating, subjects will call the study coordinator, Rory Berk, who will describe the study in more detail.

The recruitment process will start once the Protocol and all other related materials are approved by the VA commitee and the UNR IRB.

18.c. **Recruitment Materials/Invitations to Participate.**
Check all that apply and attach copies of all recruitment materials.

☒ Flyers
☒ Letters
☒ Script
☐ Emails - sent:
☐ "bcc" **or**
☐ SONA post
☐ Social media (e.g Facebook)
☐ List Serve(s)
☐ Advertisements in mass media
☒ Other → specify: Flyer posted onWPI website

☐ single recipient

SECTION V: Informed Consent

It is important to remember that informed consent is a **process** that begins with the initial contact / recruitment and continues throughout the study; informed consent is not simply the act of signing a consent form.

Please check all consent options below that apply to any or all subject populations and complete the relevant items for each:

☒ **Signed Consent (Permission) =** Written signed consent / parental permission will be obtained from subjects / parents(s) / legally authorized representative. **Complete #19 and #23.**

☐ **Waiver of Signed Consent =** Subjects / parents(s) / legally authorized representative are/is not required to sign a consent form. Subjects / parents(s) / legally authorized representative will give oral consent or permission, or investigator(s) may provide subjects / parents(s) / legally authorized representative with a written statement regarding the research, e.g., an information sheet, detailed invitation letter, introductory statement for online survey. **Complete #19, #21 and #23.**

☒ **Waiver or Alteration of Consent =** Informed consent will not be obtained from subjects / parents(s) / legally authorized representative, i.e., subjects will not know that they are (their child/ ward is) participating in a research study, or that private information about them is being used in a research study. [NOTE: Waiver of consent / parental permission is not approvable for FDA-regulated research.] **Complete # 20, #22, and #23**.

19. Consent Process

19.a. Indicate who will obtain informed consent/parental permission/assent from subjects/parent(s)/legally authorized representative.

☐ PI ☒ Research Team Member(s)
☒ Co-I(s) ☐ Other → specify: _____

19.b. Specify the language to be used by those obtaining consent/permission/assent.

19.b.i. ☒ English ☐ Spanish ☐ Other → specify: ____

19.b.ii. Describe how and by whom the translation was prepared for recruitment and consent documents that been prepared in a language other than English. Please provide a "back translated" copy of the documents.

_____

19.c. Explain when and where informed consent / parental permission / assent will be obtained from subjects/ parent(s) / legally authorized representative (e.g., clinic visit, via mail, public event, classroom).

The informed consent process will take place in private rooms at the Ioannis A. Lougaris VA Medical Center in Reno and the VASNHCS CBOCs prior to the blood draw.

19.d. Specify how long subjects / parent(s) / legally authorized representative(s) will have to consider participation.

Subjects will be told to take the time they need to decide if they wish to participate. They can have time to discuss the study with family and friends, and will have the opportunity to ask questions of the research team. Subjects may consider participation in the study until the maximum number of subjects required for the study is reached, at which time no additional subjects will be enrolled.

19.e. Describe the steps that will be taken to ensure that consent is obtained in a level of language that subjects / parent(s) / legally authorized representative will easily understand. (Specify reading level)

The consent document and all other materials (e.g. questionnaires) are written in an 8th grade reading level. In addition, subjects will be encouraged to ask questions at any time.

20. Incomplete Disclosure/Deception

☐ The study design includes the use of incomplete disclosure/deception or both. **Attach a copy of the debriefing statement.**

Describe how incomplete disclosure will be used, the rationale for using it, and how the subjects will be debriefed. NOTE: In all cases of research involving incomplete disclosure, such research is justified only if it is clear that **(1)** incomplete disclosure is truly necessary to accomplish the goals of the research, **(2)** there are no undisclosed risks to subjects that are more than minimal, and **(3)** there is an adequate plan for debriefing subjects and, when appropriate, for dissemination of research results to them [ref. Belmont Report, Part C.1.]

21. Waiver of Signed Consent

Please review the categories below to determine if this study or a portion of the study is eligible for a waiver of consent. Either Category 1 **OR** Category 2 must be true (select only one).

☐ **Category 1**

The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern.

Explain how this research meets Category 1 based on protocol specifics:

OR

☐ **Category 2**

The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

Explain how this research meets Category 2 based on protocol specifics:

Attach information sheet or script, if applicable.

22. Waiver or Alteration of Consent (Consent will not be obtained, or one or more required elements of consent will not be included in the consent process.

All must apply:

☒ The study does not pose more than minimal risk to subjects.

Explain based on protocol specifics: All subjects will sign a consent and HIPAA form in order to participate in the study. We are requesting a waiver of consent and HIPAA only for the preliminary step of identifying potential GWI subjects who meet specific criteria (group a). The research involves one blood draw (venipuncture) of approximately 30 ml (equivalent to 2 tablespoons). By definition, venipuncture is considered a minimal risk procedure. Subjects will also be asked to complete a brief questionnaire including questions related to the subject's demographic characteristics, health and medical history, and time periods and locations in which they served during the Persian Gulf War (if applicable).

☒ The waiver will not adversely affect subjects' rights and welfare.

Explain based on protocol specifics: The waiver is requested solely to identify subjects who may meet the inclusion/exclusion criteria and the information will only be used and stay within the VASNHCS following the guidelines of confidentiality and protection of protected health information (PHI). Only designated VASNHCS personnel will have access to such information. Also, once the subject agrees to participate, a normal consent process will follow, which includes having the subject read and sign a consent and HIPAA form.

☒ The research is not feasible without the waiver.

Explain based on protocol specifics: Recruitment of GWI subjects would be difficult without the waiver because the study requires subjects who meet specific criteria in order to complete the objectives of the study.

Whenever appropriate, explain how the subjects will be given additional pertinent information about the study after their participation:

☒ Appropriate → explain: The waiver is only for the purpose of identifying potential subjects. All subjects will sign a consent form and HIPAA document for actual participation in the study. If the study design or use of the data is to be changed, enrolled subjects will be informed and their consent will be re-obtained.

☐ Not appropriate → explain: _____

☐ Records, review, not appropriate.

23. HIPAA Authorization

☒ *This research is being conducted at a covered entity at the University or VASNHCS. Covered entities are defined in the HIPAA rules as (1) health plans, (2) health care clearinghouses, and (3) health care providers who electronically transmit any health information in connection with transactions for which HHS has adopted standards.*

☒ This research involves the creation, use or disclosure of protected health information. *The Privacy Rule defines protected health information (PHI) as individually identifiable health information, held or maintained by a covered entity or its business associates acting for the covered entity, that is transmitted or maintained in any form or medium (including the individually identifiable health information of non-U.S. citizens).*

If **both** statements above apply to this study, you are required to obtain separate authorization under the HIPAA Privacy Rule. Form templates and instructions are available on the UNR OHRP website (www.unr.edu/ohrp). For more information, go to the HIPAA Privacy Rule, Information for Researchers at the National Institutes of Health website (http://privacyruleandresearch.nih.gov/).

Please select the type of research subject authorization being requested:

☒ HIPAA authorization. Attach the HIPAA Authorization form.

☒ HIPAA waiver of authorization [A waiver of consent must also be requested.] Attach the HIPAA Waiver of Authorization form.

SECTION VI : Drugs/Devices, Genetic Testing, Radiation and Biological Samples

24. ☐ The proposed research involves drugs/devices.

Investigational Drug

Drug Name: _____

IND#: _____ Attach documentation from the FDA or sponsor verifying the IND number

If drug does not require IND#, please explain: _____

Attach **Drugs-Biologics and IND Exemption Checklist**.

Investigational Device

Device Name: _____

IDE# (Significant Risk device): _____ Attach documentation from the FDA or sponsor verifying the IDE number

If device does not require IDE# (Nonsignificant Risk device), please explain: _____

Attach **Device Significant Risk-Nonsignificant Risk and IDE Checklist**.

FDA-approved Drug or Medical Device

☐ The study involves the use of a FDA-approved drug or medical device.

Attach documentation from the FDA or sponsor verifying the IND/IDE number unless the IND/IDE number is included in the sponsor's protocol or investigator's brochure

Attach all of the following documents, if applicable:

☐ Clinical protocol

☐ Investigator's Brochure

☐ Sponsor Financial Disclosure form

☐ Form FDA 1572

☐ For non-VASNHCS protocols involving investigational or unlicensed test articles attach site policies and procedures for the control of test articles (unless the current version of the policies is already on file at the

University of Nevada, Reno Office of Human Research Protection)[*Please contact the UNR OHRP at 775-327-2368 if you have any questions*.]

25. ☒ The proposed research involves radiation or biological samples.

Genetic Testing

☒ The study involves the genetic testing of biological samples. Specify: Transcriptome analysis will be performed on a subset of samples to identify genetic polymorphisms. However, no specific gene will be targeted.

Radiation or Radioisotopes

☐ The study involves the use of radiation or radioisotopes in addition to what is used for standard clinical treatment.

Research cannot commence until a copy of the Radiation Safety approval letter has been submitted to the IRB.

Biological Samples

☒ The study involves the use of biological samples (either banked or prospectively obtained)? Biological samples include microorganisms; recombinant DNA; biological toxins; human blood, body fluids, tissues, and cells; nonhuman animal tissue and cells; and cell and tissue cultures.

A copy of the Memorandum of Understanding and Agreement (MOUA) approved by the UNR Institutional Biosafety Committee must be submitted to the IRB in order to initiate the approval process. MOUA and Biological Safety forms are available at http://www.ehs.unr.edu/website/.

SECTION VII: Research Plan

Please answer the following questions in language readily understandable by someone unfamiliar with the research project and outside the field of expertise. **Avoid the use of acronyms, and discipline-specific language or technical jargon, unless explained in lay terms.**

26. Introduction

Summarize the background information that led to the plan for this project. Please provide references as appropriate and, when applicable, previous work in animal and/or human studies.

The Persian Gulf War, also known as Operation Desert Storm (1990-1991), resulted in few casualties, less than 200 deaths of the 700,000 soldiers deployed. However, within months of their return to the United States, a significant number of Gulf War veterans, perhaps as many as 1/3 (175,000-210,000) of the military personnel, began to report a variety of symptoms that included fatigue, musculoskeletal discomfort, skin rashes, and cognitive dysfunction [1-4]. These symptoms collectively are known as Gulf War illness (GWI). Almost three years ago, the Research Advisory Committee on GWI presented a comprehensive report on this illness and the health of Gulf War veterans to the Secretary of Veterans Affairs [5]. Because these servicemen were subjected to a number of potentially hazardous conditions that included infectious agents, medical prophylaxis (vaccines), pesticides, smoke from oil well fires, chemical and biological warfare agents as well as psychological stress [6, 7], several hypotheses have been proposed as etiological agents.

While there is a clinical case definition applied by the Department of Defense (DOD) and Veterans Affairs (VA) for GWI, GWI does not have a clearly accepted working research case definition. Nonetheless, Fukuda et al. [3] and Haley and colleges [8] applied a factor analysis to symptoms in order to develop a working research case definition for GWI. Fuduka used the factor analysis on symptom clusters described by deployed and non-deployed military personnel and compared results to a second definition developed by a consensus of experts. On the other hand, Haley and colleagues used a case definition to define the population for factor analysis that required deployment to the theater of operations between 8/9/90 and 7/31/91, without a history of a medical or psychiatric condition which could reasonably explain the symptoms and at least 5 of the 8 symptom/signs criteria:fatigue, myalgia, arthralgia, cognitive complaints, and mood disturbance. He concluded that there was evidence of discrete subpopulations, which they separated into six subgroups: impaired cognition; confusion ataxia; arthro-myo-neuropathy; phobia-apraxia; fever-adenopathy; and weakness-incontinence.

Chronic fatigue syndrome (CFS) is the most common name used to designate a significantly debilitating medical disorder or group of disorders generally defined by persistent fatigue accompanied by other specific symptoms for a minimum of six months, not due to ongoing exertion, not substantially relieved by rest, nor caused by other medical conditions [9-11]. CFS is diagnosed as a cluster of symptoms and based on exclusion of other conditions. It is widely accepted that CFS is a heterogeneous disease with different pathophysiological disturbances that manifest with similar symptoms. Therefore, no consistently reproducible molecular biomarkers are generally accepted for the diagnosis of CFS and the development of treatment and research strategies results is difficult. Numerous studies have reported symptom profiles in GWI patients that overlap with CFS, including fatigue, pain and cognitive difficulties

(Reviewed in [12]). As has been reported in CFS, these symptoms may be of either acute or slow onset. Other studies have attempted to define immunologic abnormalities in GWI. The most convincing study to date on the immune characteristics of GWI subjects in comparison to CFS found an alteration in the expression of cytokines of individuals with GWI similar to that reported for CFS [13]. A previous review of the immunology of CFS noted that universal agreement of such cytokine abnormalities has not been achieved and disparities between reports may be largely due to difference in methodologies and differences in patient populations [14]. The CFS literature often suggests that the duration of illness impacts the severity and symptomatic profile, with shorter duration of illness, the more likely that the illness improves and resolves [15]; an observation that demonstrates the need to evaluate pathogenic mechanisms at earlier points in the disease.

A clinical description of GWI satisfies both the Center for Disease Control 1994 definition of CFS and the more rigorous 2003 Canadian Consensus Criteria [16,17]. Chemical or biological triggers have been proposed for both CFS and GWI. However, no etiological agents have been consistently described for either syndrome. As with any heterogeneous disease, it is necessary to stratify patient populations in order to identify potential biological markers and potential etiological agents. Considering GWI as a subset of patients with CFS provides the ability to stratify a subgroup of CFS patients that may have a common triggering event and more similar pathology. The identification of biomarkers in GWI may also be applicable to other subgroups of CFS patients. A significant body of research in CFS and GWI suggests an underlying innate immune dysregulation. Therefore, potential biomarkers are likely to be associated with innate immune differences between patients and controls. In order to investigate potential biochemical dysregulation in the innate immune system, and subsequently identify useful biomarkers, we will compare the white blood cell transcriptome of GWI patients to that of healthy control subjects. As applied here, the term "transcriptome" refers to the total set of transcripts observed in circulating immune blood cells. Unlike the genome, which is generally fixed for a given cell type, the transcriptome can vary as a result of external environmental conditions. Since it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time. Therefore, the transcriptome will reflect the biochemical activity of the immune cells, and in turn, the individual. This data can be compared and contrasted to that of healthy controls in order to ascertain differences particular to GWI. Additionally, transcriptome analysis may identify pathogens present in the immune cells as well as identify abnormalities in the immune biochemistry of GWI subjects. Our previous work suggests that by applying conventional statistical tests and “machine logic” algorithms to the multiple data sets of immune parameters, it may be possible to identify biomarker signatures that delineate patient populations [18].The information derived from this study may lead to a greater understanding of the pathophysiology of GWI, ultimately leading to improved patient care.

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27. Scientific or Scholarly Rationale

State the scientific or scholarly rationale for the study. What do you expect to learn from this study?

Innate immune dysregulation is a consistent observation associated with GWI. Given that a number of opportunistic microbial agents, such as mycoplasma species and human herpes virus 6 and 7, have been reported to be more prevalent in GWI compared to healthy controls, we consider that these systemic bacterial and viral infections may be important in disease onset, progression and the result of the increasing number and severity of symptoms which may contribute to the innate immune abnormalities. Therefore, because no etiological agent has been universally described in GWI, a complete characterization at a level of transcription of the innate immune response and the associated opportunistic pathogens is essential. This study may lead to a significant advancement in the understanding of GWI pathophysiology.

28. Research Questions / Purpose

What are the research questions / purpose of this study?

The purpose of the study is to explore two hypotheses. The first hypothesis is that a dysregulation of the innate immune system is associated with GWI and that it can be identified by comparing the lymphocyte transcriptome profile of GWI subjects to those of healthy control subjects. The second hypothesis is that unique opportunistic pathogens associated with GWI may contribute to the etiology of this disease and can be identified too by transcriptome analysis using next generation sequencing technology.

29. Research Methods/Study Design

What research methods will be used? Give a brief non-technical explanation. Include the study design, statistical analysis methods, and power analysis.

The study design requires the participation of a total of 200 subjects in a two-year study period (50 GWI subjects and 50 control group subjects each year. Previous reports suggest there is an up regulation of a set of 10 cytokines and chemokines in patients with similar symptoms (Lombardi et al. 2011). Using this preliminary data, we calculated the power of our study to identify the up regulation of these cytokines. Given that 96% of cases and 92% of controls were accurately differentiated using a Random Forrest generated cytokine profile in the previous study, this study with 50 GWI subjects and 50 controls, and a significance level of 0.01 (smaller than the standard 0.05) to account for multiple comparisons, still gives us a 100% statististical power to detect differences between cases and controls based on a RF generated cytokine profile. Lenth RV (2006-9). Java Applets for Power and Sample Size [Computer Software]. Retrieved February 2, 2012, from http://www.stat.uiowa.edu/~rlenth/Power.

Peripheral blood mononuclear cells (PBMCs) will be ficol separated and used for total RNA extraction. The SuperScript III CellsDirect cDNA Synthesis Kit (Invitrogen) will be used to generate cDNA. The transcriptome of 30 GWI subjects and 30 control group subjects will be initially examined by next generaton sequencing (NGS) to identify candidate sequences that will be confirmed in all 200 samples by RT-PCR. Illumina sequencing by synthesis NGS technology will be utilized to determine presence of transcripts for infectious agents as well as changes in host transcriptome. Initially, short (50 bp) nucleotide sequences will be generated complementary to the transcriptome of the sample. Generated pool of sequences will be used for genome alignment and de novo sequencing for efficient transcriptome assembly. Real time PCR analysis will be utilized to confirm identified transcripts in the remaining samples. Protein expression will be determined using western blot and ELISA. Any pathogens identified will be characterized at the level of genome, transcripts, and protein expression.

Master data will be stored using Microsoft Excel. General statistical analysis will be made using SAS 9.2 and NGS data analysis will be made using CLC genomics Workbench.

30. Procedures

Describe the study procedures, identifying which procedures are already being performed for diagnostic or treatment purposes. This should provide a detailed account (step-by-step) of what subjects will experience during their participation in the study, in the order experienced.

1. Potential GWI subjects (group a) will be contacted and recruited using a list of pre-screened subjects generated from medical records in the VASNHCS database. Dr. Hill will access the VASNHCS database to screen for eligible GWI subjects (group a) based on diagnostic ICD9 codes which define characteristic symptoms of GWI (ICD9: 780.79, ICD9: 729.1 and ICD9: 780.71 -- See list A in Procedures, Section VI, #30). Group a subjects will be excluded if their medical records indicate they have or had traumatic brain injury (TBI) or have human immunodeficiency virus (HIV). The contact list of eligible group a subjects will only include subject's name, phone number and address. A letter will be sent to the potential subject describing the study and providing a point of contact for the VA investigator and study coordinator. Unless the potential subject states they do not want to be contacted, they will receive an initial phone call. Phone calls will be made to potential subjects by any of the three recruiters using the list generated from the database. Recruiters will make a maximum of three attempt calls to contact the potential subject, leaving study coordinator contact information if the subject is not there at the moment. After three attempts, one voice mail message will be left (if available). However, information very specific to the study will not be left as a voice mail message. Subjects will have the study described to them and will be informed that in order to qualify for the study they must have been on active duty during the Persian Gulf War, but that it doesn't matter whether they were deployed to Iraq or surrounding areas for purposes of the study. They will also be provided with contact information in case they have future questions. The recruitment letter and phone script are attached.

2. Control group subjects (group b) will be recruited through flyers posted in the Ioannis A. Lougaris VA Medical Center in Reno, the VASNHCS CBOCs, local veterans' service organizations who agree to allow flyers to be posted in their areas, and the WPI website. Group b subjects will call the study coordinator if interested in participating in the study. The study coordinator will explain the study and screen potential subjects for eligibility, if subjects are interested in participating. The screening script is attached.

3. All subjects (group a and b) will be told that this study is being done to better understand what causes GWI by looking at differences in the immune system composition between veterans who have GWI and veterans or non-veterans who don't have it. They will be told that the study requires both a blood sample of 30 ml (about 2 tablespoons) and the completion of a participant questionnaire including questions related to the subject's military service as it relates to the Persian Gulf War (if applicable); demographic characteristics such as age, gender and location; and health and medical history. They will be informed that they can take time to discuss the study with friends or family prior to agreeing to participate, and that they can stop participating at any time during the study. They will also be told that if they choose not to participate in this study, it will not have any effect on or change any care they would normally receive or are eligible to receive through the VA or or any other medical care provider. They will be informed that if they participate in the study, they will receive a $25 check to compensate them for their time/travel in connection with the study.

4. The study coordinator will schedule appointments for subjects to sign informed consent and HIPAA authorization forms and complete the questionnaire and blood draw. At this appointment, subjects will be given sufficient time to carefully read the consent and HIPAA forms prior to signing. Subjects will also be informed that they will receive a $25 check from Sierra Veterans Research and Education Foundation to compensate them for their time/travel in connection with the study, and that their name, address and social security number will be collected for purposes of processing payment. They will again be informed that that they can stop participating at any time during the study and that it will not have any effect on or change any care they would normally receive or are eligible to receive through the VA or any other medical care provider.

5. The participant questionnaire and blood samples will be coded (de-identified) at the time of collection. All data collection instruments will remain properly secured at the VA in locked file cabinets and locked offices of the study coordinator and/or Dr. Hill's office. Samples drawn at the Ioannis A. Lougaris VA Medical Center laboratory in Reno or a laboratory at the VASNHCS will be picked up by an IATA-certified courier and delivered to WPI or shipped overnight to WPI by a courier service with IATA certification. Samples collected in a laboratory at a CBOC will be shipped overnight to WPI by a courier service with IATA certification.

List A. ICD9 Codes

ICD9: 780.79 includes the following symptoms:

- Weakness; lack of energy and strength.
- Physical weakness, lack of strength and vitality, or a lack of concentration.
- Exhaustion that interferes with physical and mental activities.
- State of weariness following a period of exertion, mental or physical, characterized by a decreased capacity for work and reduced efficiency to respond to stimuli.
- An overwhelming sustained sense of exhaustion and decreased capacity for physical and mental work at usual level

• That state, following a period of mental or bodily activity, characterized by a lessened capacity for work and reduced efficiency of accomplishment, usually accompanied by a feeling of weariness,

ICD9: 729.1 includes the following symptoms:

• An acute, subacute, or chronic painful state of muscles, subcutaneous tissues, ligaments, tendons, or fasciae caused by a number of agents such as trauma, strain, occupation, exposure, posture, infection, or arthritis.
• Inflammation and fibrous degeneration of a muscle.
• A common nonarticular rheumatic condition that is characterized by muscle pain, tenderness, and stiffness.

ICD9: 780.71 includes the following symptoms:

• Distinctive syndrome characterized by chronic fatigue, mild fever, lymphadenopathy, headache, myalgia, arthralgia, depression, and memory loss; candidate etiologic agents include Epstein-Barr and other herpesviruses.
• Syndrome thought to be caused by a viral organism resulting in chronic fatigue, fever, pain, sore throat, and, in some cases, depression.
• A syndrome of unknown etiology. Chronic fatigue syndrome (CFS) is a clinical diagnosis characterized by an unexplained persistent or relapsing chronic fatigue that is of at least six months' duration, is not the result of ongoing exertion, is not substantially alleviated by rest, and results in substantial reduction of previous levels of occupational, educational, social, or personal activities. Common concurrent symptoms of at least six months duration include impairment of memory or concentration, diffuse pain, sore throat, tender lymph nodes, headaches of a new type, pattern, or severity, and nonrestorative sleep. The etiology of CFS may be viral or immunologic. Neurasthenia and fibromyalgia may represent related disorders. Also known as myalgic encephalomyelitis.

31. Time Commitment for Subjects
Describe the total time commitment for subjects. If subjects are expected to participate on multiple occasions, the time for each occasion in addition to the cumulative duration should be included.

The total amount of time commitment for the subjects can vary between approximately 25 minutes to 40 minutes. The participant questionnaire should take no more than approximately 5 minutes to 10 minutes. Having the blood drawn, including potential wait time, should take no more than approximately 20 minutes to 30 minutes.

32. Withdrawal
Describe the plan for voluntary and involuntary withdrawal of subjects in the study, if applicable.

Subjects can withdraw from the study at any time. Withdrawing from the study will not have any effect on any care they are eligible to or normally receive through VASNHCS or any other medical care provider. Involuntary withdrawal will only occur if a subject does not complete both requirements of the study: blood sample and completion of the participant questionnaire. For both cases (voluntary and involuntary withdrawal), samples will be discarded following standard operational procedures, and all data collection instruments (stored at the VASNHCS) will be destroyed in accordance with VA Record Control Schedule.

33. Web-based Survey

☐ A web-based survey management provider (commercial or private) will be used for this project. The URL for the survey is: _____

Subjects will receive the URL by: (**Check all that apply)**

☐ Researchers will email subjects the survey URL.
☐ Researchers will email subjects the survey URL and retain the ability to associate subjects' responses with emails/names.
☐ Researchers will have the survey management provider email the survey link to subjects on their behalf.
☐ Researchers will post the URL for the survey to a website(s).
☐ Other → explain: _____

34. Study Instruments
List and **attach** each questionnaire, survey, diary, assessment, and measurement. **Describe** the purpose and use of each, cite the source, and indicate whether copyrighted.

1. Participant questionnaire: The purpose of this questionnaire is to collect certain information from all subjects (group a and b) and includes questions related to the subject's demographic characteristics such as age, gender and location; health and medical history; and information about the subject's military service as it relates to the Persian Gulf War, if applicable.

2. Recruitment phone script: The purpose of this script is to provide recruiters with a standard tool to recruit potential GWI subjects (group a).

3. Recruitment letter: The purpose of this letter is to recruit potential GWI subjects (group a) to participate in the study.

4. Sreening script: The purpose of the script is to provide the study coordinator a standard tool to screen potential healthy subjects (group b) for the study. (There is no screening script required for group a subjects because they will be pre-screened using the database as described herein.)

35. Videotaping, Audio Taping, and/or Photographs

☐ Audio taping will be used in this project. Describe the purpose and use of audio taping. _____

☐ Videotaping and/or photography will be used in this project. Describe the purpose and use of videotaping and/or photographs. _____

Attach Video/Photograph Consent form.

36. Payment / Compensation / Incentives

☒ Payment / compensation / incentives (including course credit) will be given to subjects in this project.
IMPORTANT: Providing payment / compensation / incentives to subjects cannot be contingent upon their completion of the study.

☐ Compensation is psychology research experience or social psychology research credits. Standard distribution apply.

☐ One per hour (survey research)
☐ Two per hour (lab research)

☒ Compensation is **payment / compensation / incentives** (e.g. monetary, free services, gifts, course credit, or extra credit) that will be given to subjects. Explain the payment arrangements (e.g. amount and schedule of payment and the proposed method of disbursement), including reimbursement of expenses. Subjects who qualify for the study will receive a $25 check through the Sierra Veterans Research and Education Foundation. Subject's name, address, and social security number will be given to this entity so that the subject's payment can be processed.

☐ Payment / compensation / incentives for this project will originate from UNR-administered grants or contracts. IMPORTANT: The UNR Controller's Office requires identifying information from subjects to issue checks, cash, or gift certificates to payees originating from UNR-administered grants or contracts. Please explain how identifiable subject information will be handled: _____

☐ There will be partial payment (proration) if the subject withdraws prior to completion of the study.

☐ Course or extra credit are offered and students will be given alternative activities that are equivalent in time and effort to the research participation and provide the same amount of credit.

Please justify the proposed payment arrangements. Include how the proposed payment does not present undue pressure (or coercion) to for the subjects to participate. The payment is a nominal amount to compensate subjects for their travel and time involvement required for the blood draw and completion of the participant questionnaire. The payment is not significant enough to present undue pressure for the subjects to participate.

☐ The research involves the possibility of added expense (**costs**) to the subjects or to a third party (such as an insurer), longer hospitalization, extra laboratory tests, travel, time missed from work.

Specify what the sponsor will cover and/or how the subjects will be compensated. (Note: Time is not considered a cost to subjects.) _____

☒ Veteran subjects will be recruited.

Provide information regarding Department of Veterans Affairs coverage of subject costs incurred as the result of problems/adverse events that may arise during their participation in this study. The Department of Veterans Affairs

will provide necessary medical treatment at the VA medical facilities for research subjects injured by participation in a research study under the supervision of one or more VA employees, except in limited circumstances. Exceptions include: situations where VA facilities are not capable of furnishing economical care; situations where VA facilities are not capable of furnishing the care or services required; and situations involving a non-veteran research subject. This does not apply to treatment for injuries that result if the subject does not comply with study procedures. Study subjects will be responsible for any expense incurred such as travel and lost time from work.

☒ This research may lead to the development of a commercial product.

Specify whether or not the subject will be compensated for the sale of the product(s). If this research leads to the development of commercial products or discoveries that could be patented, registered, or otherwise developed for commercial sale, subjects will not receive any financial benefit from that. Subjects will not have patent or ownership rights to any products or discoveries resulting from this research.

SECTION VIII: Risks and Benefits

37. Risks and Inconveniences

37.a. Identify the risks to subjects.

	LIKELIHOOD		LEVEL OF RISK		IMPACT
	Unlikely	Likely	Minimal	Greater than Minimal	Describe Risk (e.g. bruising, etc.)
Physical	☒	☐	☒	☐	Occasional fainting (seldom happens), bruising and/or infection at the site of the needle stick (extremely rare)
Psychological	☒	☐	☒	☐	Some subjects may experience psychological distress in response to the questionnaire
Social	☐	☐	☐	☐	Unknown
Legal	☐	☐	☐	☐	Unknown
Financial	☐	☐	☐	☐	Unknown
Employment	☐	☐	☐	☐	Unknown
Information/Privacy	☒	☐	☒	☐	Loss of privacy/confidentiality
Other, _____	☐	☐	☐	☐	_____
Other, _____	☐	☐	☐	☐	_____
Other, _____	☐	☐	☐	☐	_____

Additional information:

37.b. Describe the steps to be taken to minimize each risk identified above.

The following measures will be taken to minimize each of the risks described above:

1. Physical discomfort of blood draw: Blood will be drawn at the Ioannis A. Lougaris VA Medical Center in Reno or the VASNHCS CBOCs by an experienced phlebotomist.

2. Psychological distress: Subjects experiencing psychological distress related to their participation in this research can choose not to participate in the study and can withdraw at any time. If deemed necessary veteran subjects will be provided with a referral to VA mental health services.

3. Social: We do not expect any social distress.

4. Legal: We do not expect any legal distress.

5. Financial: We do not expect any financial distress.

6. Employment: This study does not involve any employers of the participants, and we do not expect any employment issues nor will we share any personal/individual information of any participant obtained in this study with any employer.

7. Information/Privacy Loss: All participant questionnaires and blood samples will be coded. Dr. Hill will maintain a copy of the coded list that matches subjects' names to their code number. That code list will be secured on a VASNHCS secure network and a hard copy in a locked file cabinet in Dr. Hill's office at the VA Research Building. Only Dr. Hill, Dr. Young, and study coordinator, Rory Berk, will have access to this master code. The office will be locked any time one of these research members is not in the office. In addition, all participant questionnaires and consent forms will remain at the VASNHCS and will be stored in different key locked file cabinets. Only coded blood samples will be sent to WPI. The samples will be stored in locked freezers in the WPI research lab, which is locked at all times and only accessible by key card. Only data from the participant questionnaire will be sent coded (de-identified) to the WPI and will be stored in the WPI database identified by that number. The University of Nevada, Reno is the Institution providing the IRB review under the IRB Registration # IRB00000215 and Federal Wide Assurance (FWA# FWA00002306). VASNHCS holds FWA00002304 and lists both UNR IRB and VA Central IRB as IRBs of record. WPI holds FWA00014406 and lists UNR IRB as IRB of record. All research personnel have completed the appropiate CITI and VA training and will maintain required research related education through VASNHCS and UNR IRB.

38. **Research with Greater than Minimal Risk.**

38.a. Describe the provisions for monitoring data to ensure the safety of the subjects (e.g. for greater than minimal risk research).

_____

38.b. If medical or psychological services are needed as a consequence of the research, describe how the subject will be referred to those services.

Every reasonable safety measure will be used to protect subjects' well-being. If subjects are injured as a result of taking part in this study, the VA will provide necessary medical treatment at no cost to the subject unless the injury was due to the subject not following the study procedures.

39. Benefits

39.a. Describe potential benefits to science, society, or a specific class of individuals. Include the importance or value of the knowledge this study is likely to generate.

New knowledge about the possible relationship between GWI and bacterial-viral infection and immune dysfunction may lead to novel therapeutic strategies for those affected by GWI.

39.b. Describe potential direct benefits to individual subjects, if any. **Do not include** any incentives (money, gifts, etc.) here.

☐ Potential benefit → explain: _____

☒ None anticipated

40. Risk/Benefit Analysis

☒ Minimal risks; some potential benefits.

☐ Risks are greater than minimal but justified by the benefits.

☐ Risks outweigh the benefits to the subjects. Explain why the research should be conducted._____

SECTION IX: Privacy and Confidentiality

41. Privacy

Privacy involves the right of individuals to control access to their person, behavior, viewpoints, and private identifiable information.

How will the investigators protect the privacy of subjects at the time of recruitment, and during and after participation? The response should discuss how subjects will be recruited, including how researchers obtain initial contact information (if applicable) and when and where study participation takes place. For example, does recruitment and subject participation require a private setting rather than a public space?

The researchers are requesting waivers of informed consent and HIPAA for the purpose of identifying potential GWI subjects (group a) from the VASNHCS database. The list of potential subjects will be limited to name, telephone number and address. Group a subjects contacted as a result of the list generated from the database will receive a letter describing the study and saying that someone will contact them by phone at a later date to ask them if they are interested in the study, if they have any questions, and if they would like researchers to find out if they are eligible to participate. Three attempts will be made to contact the veteran by phone, but no specific study information will be left as a message. Healthy (group b) subjects will make the first contact to the study coordinator, Rory Berk, as a result of seeing flyers describing the research study. The study coordinator will describe the study to these potential participants and answer any questions. If group b subjects are interested in participating, the study coordinator will use a screening script to determine their eligibility. Subjects who agree to participate will sign informed consent and HIPAA forms prior to participation in the research. The consent process, completion of the participant questionnaire, and blood draw will be done in private rooms at the Ioannis A. Lougaris VA Medical Center in Reno or the VASNHCS CBOCs. These rooms have doors that seclude subjects from all others, maintaining their privacy and confidentiality. In addition, all data collection instruments will be coded (de-identified), properly secured, and remain within the VA. Only coded data collected from the participant questionnaire and coded blood samples will be sent to the WPI and securely stored according to standard operational procedures. The coded data will be maintained in a secured electronic database on the PI's computer. The PI's computer is located in the PI's office in room L303A of the CMM.

☐ For online surveys, subjects will be told to close the web browser following completion of the survey in a public location or to delete cookies from their home computer.

If a web-based survey management provider will be used, please provide a copy of the site's privacy policy.

42. Confidentiality: How will researchers protect the confidentiality of information collected from or about subjects to ensure that it is not disclosed other than as described in this application? All data will be highly protected by the research team, all of whom have taken the required CITI and VA trainings that emphasize the protection of subjects' privacy and confidentiality. Only assigned research personnel will access the data. Subjects' identities will not be revealed to third parties in any publications or at any time or any place during or after this project. Subjects are given random numbers with no personal identifiers and only data collected from the participant questionnaire will be stored in the WPI database identified by that number. The participant questionnaire will be kept secured at the VA. A master code will be maintained that links each subject to a number. That list will be maintained in Dr. Hill's office in a locked file cabinet or as a secured electronic database. Only Dr. Hill, Dr. Young, and Rory Berk will have the ability to access the master code. All study data (including data collected from participant questionnaire) will be coded. Serum samples will be coded (subject identifiers removed) prior to sending samples to the WPI lab.

41.a. Please provide the location where data will be stored. Consent forms and master code sheets must be stored separately from data.

The master code list will be secured on a VASNHCS secure network and a hard copy in a locked file cabinet in Dr. Hill's office at the VA Research Building. In addition, the consent forms and participant questionnaires will remain at the VASNHCS and will be stored in different key locked cabinet files designated by Dr. Hill. The office will be locked any time Dr. Hill or one of the research team members is not in the office. Only coded data and coded blood samples will be sent to WPI. The coded data will be maintained in a secured electronic database on the PI's computer. The PI's computer is located in the PI's office in room L303A of the CMM. Coded samples will be kept secured at all times in two locked -70 deg. freezers and 2 LN2 (liquid nitrogen) tanks in WPI's lab room L-300 of the CMM.

42.b. Please state how long data will be stored locally.

Study data will be stored locally for the duration of the study and in accordance with VA Record Control Schedule, a minimum of two (2) years following publication. Samples will be stored in the WPI laboratory for the same duration. Study data retained in the VASNHCS will be destroyed in accordance with VA Record Control Schedule. No biological samples will be retained in the VASNHCS or WPI following completion of the research.

42.c. If data are collected through host survey management system, please state how long data will reside at the site.

_____

42.d. Describe how data will be downloaded from the host server with respect to proposed security measures, and whether the data will have any associated identifiers (email and/or IP addresses).

_____

42.e. Please also state how long data will reside on the host server prior to deletion, if the site server and data server are different.

_____

42.f. Please describe what will happen to all study-related data after the storage period elapses.

- ☒ All data (electronic or hard copy) will be destroyed.
- ☐ All audiotapes will be erased or destroyed.
- ☐ All videotapes will be erased or destroyed.
- ☐ Other→ Describe: _____

42.g. If audio taping and/or transcription will be used, please describe how subjects' identities will be protected (use of pseudonyms or avoidance of names).

- ☐ Pseudonyms will be used in recordings and/or transcriptions.
- ☐ Use of names will be avoided during recording and/or transcriptions
- ☐ Names used in tapes but not in transcriptions
- ☐ Names used but identity protected by _____
- ☐ Other→ Describe: _____

42.h. ☒ Limits to confidentiality exist. Explain: _____

Indicate by checking the appropriate boxes below who will have access to the study records / data, e.g. investigators, research assistants, advisors, and external agencies (e.g., study sponsors, collaborating institutions, regulatory agencies).

IMPORTANT: For the purpose of regulatory oversight, the University of Nevada, Reno Institutional Review Board, the federal Office for Human Research Protections, and the Food and Drug Administration (FDA) (for FDA research) will have access to the study records / data.

Check all that apply:

- ☒ Principal Investigator/Faculty Advisor
- ☒ Research Team Member
- ☒ Study Sponsor
- ☒ Collaborating Organizations: specify: → The VA Sierra Nevada Health Care System
- ☐ Other: specify: → _____

42.i. ☐ Sensitive information (e.g. illegal drug use, criminal activity) will be collected about subjects and maintained. Indicate whether or not a Certificate of Confidentiality will be obtained. (See the NIH Certificates of Confidentiality Kiosk at http://grants.nih.gov/grants/policy/coc/index.htm for further information.

- ☐ Yes. Please provide a copy to IRB upon receipt.
- ☐ No. Please explain why not: _____

42.j. ☒ Data will be coded (names of subjects replaced with codes).
Explain all coding procedures. NOTE: Personal identifiers or portions of personal identifiers may only be used for coding purposes if these identifiers could not reasonably be linked to a specific individual.

Data will be coded by using a finite series of numbers generated and their order will be randomized. The numbers will not contain any personal identifiers. A unique number will be assigned to each subject and only that number will be used to identify questionnaires, biological samples, and study data. Study records and the master code will be maintained securely in separate key file cabinets in the VASNHCS in Dr. Hill's office. Only Dr. Hill, Dr. Young, and study cordinator, Rory Berk, will have the ability to access the master code. WPI researchers will never have access to any data that can link a subject with a given biological sample or questionnaire.

Section X: Assurances

Principal Investigator Assurance

I hereby certify that the study procedures described in the attached protocol have been designed, to the best of my ability and knowledge, to protect human subjects engaged in research in accordance with the standards set by University of Nevada, Reno, the United States Department of Health and Human Services, the Food and Drug Administration (when appropriate), the Department of Veterans Affairs (when appropriate), and any other sponsoring federal agency.

I agree to accept responsibility for the scientific conduct of the research involving human subjects and to provide information and/or progress reports to the University of Nevada, Reno Institutional Review Board as required. I verify that all researchers are appropriately credentialed to do the services provided and the work undertaken in this protocol.

I further certify that my participation and the participation of any co-investigators does not, in any way, violate the University of Nevada, Reno policy on conflicts of interest.

Principal Investigator: _____ Date _____

Student: _____ Date _____
(Required for student-initiated research)

Responsible Official Assurance

I hereby confirm that this protocol application is scientifically sound and has scholarly merit; the researcher(s) are qualified to conduct this research and protect the research subjects; and the investigator(s) have the resources needed to protect research subjects and adequately pursue and complete the project.

Responsible Official: _____Date _____
[**Signature only required for initial submission**. This individual should be the Department Chair, Program Director, or dean in charge of the administrative unit to which the PI reports. **Neither the PI nor any other member of the research team may sign as the Responsible Official.**]

University of Nevada, Reno

Office of Human Research Protection
218 Ross Hall / 331, Reno, Nevada 89557
775.327.2368 / 775.327.2369 fax
www.unr.edu/ohrp

Certification of Approval for Modifications
Biomedical Institutional Review Board
FWA00002306

Date: September 11, 2012
To: Vincent C Lombardi, PhD Department of Pathology and Laboratory Medicine
Copy: Research Office VASNHCS
Craig Ballard

UNR Protocol Number: B12-036
Protocol Title: Pathogen and Biomarker Discovery in Gulf War Illness
Sponsor Names: US Department of Defense, US Department of Defense

Type of Review: Expedited 2, 5 & 7 Minimal risk

Meeting/Review Date: 09/11/2012
Approval Period: June 26, 2012 to June 25, 2013

This approval is for:
Approved number of subjects: 200
Approved documents:
Modified Flyer – version 3
Invitation Letter – version 3
Telephone Script
Flyer version 3, Invitation letter version 3, and telephone script have all been changed to clarify the inclusion/exclusion criteria regarding symptoms of Gulf War Illness syndrome. In addition, the telephone script was modified to include a change of phone number for the study coordinator.

The above-referenced protocol was reviewed and approved by one of UNR's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56).

Problems Researchers Must Report to the Research Integrity Office or IRB Staff
(to be reported as soon as possible, but within 10 business days)

- New or additional risks: Outcomes that the principal investigator believes are unexpected, related to the research, and suggest the research may place participants or others at greater risk of harm than was previously known or recognized
- Changes to expected harms or benefits: Any report indicating the frequency or magnitude of harms or benefits may be different than initially presented to the IRB
- Privacy: Any invasion of privacy related to an individual's participation in research
- Confidentiality: Any breach of confidentiality involving research data
- FDA Changes: Any change in FDA labeling or approval for a drug, device or biologic used in a research protocol
- Immediate harm: Any change to the protocol to eliminate an apparent immediate hazard to a research participant, prior to seeking IRB review and approval
- Prisoner: Any incarceration of a participant in a protocol not approved to enroll prisoners
- Sponsor: Any event that requires prompt reporting to the sponsor

- Sponsor: Any sponsor-imposed suspension for risk
- Protocol change: Any accidental or unintentional change to the IRB approved protocol that harmed participants or others, indicates participants or others may be at increased risk of harm, or has the potential to recur
- Device: Any unanticipated adverse device effect
- Department of Health: Any non-compliance identified by Department of Health audit or monitoring
- Federal agency: Any investigation or report by federal agency related to the research
- Medical license or practice changes: Any loss of license or hospital privileges by any researcher on the study
- Complaints: Any complaints that suggest participants or others may have been harmed or placed at increased risk of harm

PI Responsibilities

- Maintain an accurate and complete protocol file.
- Submit continuing projects for review and approval prior to the expiration date.
- Submit proposed changes for review and approval prior to initiation, except when necessary to eliminate apparent immediate hazards to subjects. Such exceptions must be reported to the IRB at once.
- Report any unanticipated problems which may increase risks to human subjects or unanticipated adverse events to the IRB within 5 days.
- Submit a closure request 10 days after project completion to the IRB.

Reference the protocol number on all related correspondence with the IRB. If you have any questions, please contact Rebecca Thomas at 775.327.2368.

For Veteran's Administration research only

VA Research: Yes
Flag VA Medical Record: No

Department of Veterans Affairs

Memorandum

Date: July 26, 2012

From: Associate Chief of Staff for Research, VASNHCS, Reno, NV (654/151)

Subj: Memorandum of Approval for New Study

To: Vincent Lombardi, Ph.D., Principal Investigator (654/151)

Re: Research Study Title: **Pathogen and Biomarker Discovery in Gulf War Illness**
ePROMISe ID#: 1164401
IRB#: B12-036
RSS Approval Date: June 26, 2012
PRS Approval Date: July 03, 2012
IRB Approval Date/Level of Review: June 26, 2012/Expedited 2, 5 & 7 Minimal Risk
RDC Approval Date: July 26, 2012
Enrollment # (current/maximum): 00/200
IRB Initial Approval Date: June 26, 2012
Research Expiration date: June 25, 2013

1. The Research Office has received the required approval recommendations from the Research and Development Committee (RDC) and its subcommittees, which include the Protocol Review Subcommittee (PRS), Institutional Review Board (IRB), and the Research Safety Subcommittee (RSS), you must retain this memorandum of approval with your research files. Your research request has been approved.

2. Any changes in your research protocol require that you submit a modification request to the IRB through the Research Office. If you need to enroll more subjects than approved by the RDC/IRB, you must submit a modification request to increase the approved enrollment number and provide justification for the increase. Any changes in your research protocol require that you submit a modification request to the IRB through the VASNHCS Research Office. To continue your research after the expiration date, you must contact the Research Office at least **two** months prior to the Research expiration date listed above.

3. As the Principal Investigator (PI) you are required to maintain all training requirements for your research team members throughout the study. Please ensure that you and your team members stay current with the Research Service required courses (listed below) and that all training documentation is forwarded to the Research Office in a timely manner. This should include:

   A. 1 - course required every two years by ORD Collaborative IRB Training Initiative (CITI) via www.citiprogram.org :
   Human subjects Protection & Good Clinical Practices (HSP&GCP)

   B. 3 - courses required annually by VASNHCS (via TMS):
   VA Privacy and Information Security Awareness and Rules of Behavior; VHA Privacy Policy Web; and Ethics Most Wanted Training

   C. 1 - course required on a one-time basis by OI&T (via TMS):

Information Security 201 for Research & Development Personnel

4. Department of Veterans Affairs policy strongly encourages VA professionals to publish scientific papers, provide scientific exhibits, and participate in other scientific communications. VA Sierra Nevada Health Care System (VASNHCS) policy requires that your role and any support from the Department of Veterans Affairs be acknowledged in any publication, exhibit, report or presentation resulting from your research. Publications and presentations must be approved through the research office prior to their submission for publication or presentation.

5. On behalf of the entire VASNHCS Research and Development Department, I wish to thank you for your interest and efforts in conducting quality research at the VASNHCS and wish you continued success on your project.

Elizabeth E. Hill

Elizabeth E. Hill, PhD, RN
ACOS for Research

cc: Research Office
Pharmacy

Certification of Approval for Waiver of HIPAA Authorization
Biomedical Institutional Review Board

Date: June 26, 2012
To: Vincent C Lombardi, PhD Department of Pathology and Laboratory Medicine
Copy: Research Office VASNHCS

UNR Protocol Number: B12-036
Protocol Title: Pathogen and Biomarker Discovery in Gulf War Illness
Type of Review: Expedited 2, 5 & 7 Minimal risk
Approval Period: June 26, 2012 to June 25, 2013

The IRB approved a request for a waiver of the requirement to obtain HIPAA authorization to access and use protected health information/patient medical record information. In granting approval of this waiver request, the IRB Chair/Vice Chair determined, based on an evaluation of the research procedures and the waiver justification submitted by the principal investigator, that all the following HIPAA waiver criteria were met. Access to the respective medical record information is permissible due to the investigator's job responsibilities in providing direct health care to the respective patients.

HIPAA Waiver Criteria

1. The use or disclosure of protected health information involves no more than minimal risk to the privacy of individuals, based on, at least, the presence of the following elements:

   ☒ an adequate plan to protect the identifiers from improper use and disclosure;

   ☒ an adequate plan to destroy the identifiers at the earliest opportunity consistent with the conduct of the research, unless there is a health or research justification for retaining the identifiers or such retention is otherwise required by law; and

   ☒ an adequate written assurance that the protected health information will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of protected health information would be permitted (i.e., under the HIPAA regulations).

2. The research (research activity) could not practicably be conducted without the waiver or alteration.

3. The research (research activity) could not practicably be conducted without access to and use of the protected health information.

PI Responsibilities

☒ Maintain an accurate and complete protocol file.
☒ Submit continuing projects for review and approval prior to the expiration date.
☒ Submit proposed changes for review and approval prior to initiation, except when necessary to eliminate apparent immediate hazards to subjects. Such exceptions must be reported to the IRB at once.
☒ Report any unanticipated problems which may increase risks to human subjects or

unanticipated adverse events to the IRB within 5 days.

☒ Submit a closure request 10 days after project completion to the IRB.

Reference the protocol number on all related correspondence with the IRB. If you have any questions, please contact Gwenn Snow at 775.327.2368.

For Veteran's Administration research only

VA Research: Yes

Flag VA Medical Record: No

Gwenn Snow, MS, RD

Office of Human Research Protection

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MD 21702-5012

REPLY TO
ATTENTION OF

MCMR-RP

1 AUGUST 2012

MEMORANDUM FOR THE RECORD

SUBJECT: Initial Approval for Protocol, "Pathogen and Biomarker Discovery in Gulf War Illness," Submitted by Vincent Lombardi, PhD, Whittemore Peterson Institute for Neuro-Immune Disease, Reno, Nevada, Proposal Log Number GW100091, Award Number W81XWH-11-1-0766, HRPO Log Number A-16878

1. The subject protocol (application dated 30 May 2012) was approved by the University of Nevada Reno (UNR) Institutional Review Board (IRB) on 26 June 2012, and by the VA Sierra Nevada Health Care System (VASNHCS) on 26 July 2012. This protocol was reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, U.S. Army and USAMRMC human subjects protection requirements.

2. This no greater than minimal risk study is approved for enrollment of 200 subjects.

3. The Principal Investigator has a duty and responsibility to foster open and honest communication with research subjects. The USAMRMC strongly encourages the Principal Investigator to provide subjects with a copy of the research protocol, if requested, with proprietary and personal information redacted as needed.

4. The following are reporting requirements and responsibilities of the Principal Investigator to the HRPO. Failure to comply could result in suspension of funding.

a. Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc), significant change in study design (i.e. would prompt additional scientific review) or a change that could potentially increase risks to subjects.

b. All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (301-619-2165), by email (HRPO@amedd.army.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

c. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the Institutional Review Board (IRB), the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

d. A copy of the continuing review approval notifications by the UNR IRB and VASNHCS must be submitted to the HRPO as soon as possible after receipt of approval. According to our records, it appears the next continuing review by both IRBs is due no later than 25 June 2013. Please note that the HRPO also conducts random audits at the time of continuing review and additional information and documentation may be requested at that time.

e. The final study report submitted to the UNR IRB and VASNHCS, including a copy of any acknowledgement documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.

f. The knowledge of any pending compliance inspection/visit by the Food and Drug Administration (FDA), Office for Human Research Protections, or other government agency concerning this clinical investigation or research; the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions; and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.

5. Please Note: The USAMRMC ORP HRPO conducts random site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

6. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project.

7. The HRPO point of contact for this study is Stephanie Mizell, RN, MPH, CIP, Human Subjects Protection Scientist, at 301-619-1032 or Stephanie.mizell@us.army.mil.

DONAHUE.SARAH.LOUISE.1299298929

Digitally signed by: DONAHUE.SARAH.LOUISE.1299298929
DN: CN = DONAHUE.SARAH.LOUISE.1299298929 C = US O = U.S. Government OU = DoD
Date: 2012.08.01 17:20:39 -05'00'

SARAH L. DONAHUE, PHD, MPH, CIP
Human Subjects Protection Scientist
Human Research Protection Office
Office of Research Protections

DEPARTMENT OF VETERANS AFFAIRS
VA Sierra Nevada Health Care System
Ioannis A. Lougaris VA Medical Center

In Reply Refer to: 654/151

Date:

Name
Address
City, State Zip

Dear:

My name is Elizabeth Hill, PhD, RN. I am a member of a research team at the VA Sierra Nevada Health Care System in Reno (VASNHCS-Reno). Our study coordinator, Rory Berk, would like to call you regarding the Clinical Research Study titled: ***Pathogen and Biomarker Discovery in Gulf War Illness (GWI)***, and your being a potential participant for the study. The pupose of the research study is to investigate the causes of GWI. Many people who were in the military and served in the Gulf War became ill and reported having symptoms that were common among them; these symptoms became known as GWI. However, we are still not sure what causes the illness. We hope that this study will help us to understand what causes it.

Diagnoses of traumatic brain injury (TBI) or human immunodeficiency virus (HIV) will prevent you from participating in this study. If you qualify to participate in the study, you will be asked to complete a participant questionnaire (5-10 minutes); it includes questions related to your military and demographic characteristics, your health and medical history, and the time periods and locations in which you served during the Persian Gulf War. You will also be asked to provide a blood sample, about two tablespoons (30 ml) drawn by an experienced phlebotomists in VASNHCS. We will pay you a small travel/time stipend if you qualify to participate in this study.

The research study will be performed at the VA Sierra Nevada Health Care System's Ioannis A. Lougaris VA Medical Center in Reno and the Community Based Outpatient Clinics where the consent process and blood draw will take place, as well as the Whittemore Peterson Institute where blood samples will be processed and analyzed.

In this study, researchers will be looking at differences in the immune system composition of veterans who served during the Persian Gulf War (Desert Storm: 1990-1991) and who have symptoms of GWI in comparison to subjects (veterans or non-veterans) who don't have symptoms of GWI. Taking part in a research study is ultimately your decision. You do not have to agree to participate if you don't want to. If you decide to participate, you also have the freedom to withdraw from the study at any time. Your decision to participate in the study, not to participate, or to withdraw will not affect the medical care you normally receive or are eligible to receive at the VASNHCS-Reno. If you have any questions about your rights as a research participant, you may contact the Office of Research Compliance VASNHCS at 775-328-1177.

Thank you for your consideration. You will receive a phone call within the next two weeks to see if you would like to participate and to answer any questions you may have about the study. If you have any questions, please call me at 775-328-1752, or e-mail me at Elizabeth.hill4@va.gov. You can also contact the study coordinator, Rory Berk at 775-328-1750. We look forward to speaking with you.

With kind regards,
Elizabeth E. Hill, PhD, RN VASNHCS
Associate Chief of Staff/Research

Ioannis A. Lougaris
VA Medical Center
1000 Locust Street
Reno, Nevada 89502-2597
(775) 786-7200 or
(888) 838-6256

VA Sierra Foothills
Outpatient Clinic
11985 Heritage Oaks Place
Auburn, California 95603
(530) 889-0872 or
(888) 227-5404

VA Carson Valley
Outpatient Clinic
925 Ironwood Drive
Suite 2102
Minden, Nevada 89423
(775) 782-5265

VA Lahontan Valley
Outpatient Clinic
345 West A Street
Fallon, Nevada 89406
(775) 428-6161 or
(866) 504-0490

Winnemucca Rural
Outreach Clinic
(877)320-4990

VA Diamond View
Outpatient Clinic
110 Bella Way
Susanville, California 96130
(530)251-4550 or
(877)816-8572

FOLLOW UP PHONE CALL SCRIPT

Interviewer initials: _____
Date: _____

Participant's name: _____
Phone Number: _____

Call Attempts Date/Time_____, _____, _____

Message left Date/Time _____

Mr./Mrs._____, 3 weeks ago we sent you an invitation letter describing the research study titled **Pathogen and Biomarker Discovery in Gulf War Illness.** This phone call is to verify that you received that letter, and to answer any questions you may have about the study, and to ask you if you would like researchers to find out if you are eligible.

Would you like researchers to find out if you are eligible? Yes _____No_____

If Yes, schedule appointment with Dr. Davis to see if subject meets the inclusion criteria.

If No, say Thank You and Good-bye.

If Not Sure and would like more information about the study: provide phone number of either: Vincent Lombardi, PhD (775-682-8278), Elizabeth Hill, PhD (775-328-1752) or Rory Berk, Study Coordinator (775-328-1750).

II. When talking to families on the phone:

Hello! This is (Full Name). Is (Name of the person) available?

If P is not available, ask person on the phone: Is there a good time to reach him/her?

If asked what this call is about, tell the person on the phone: I am with the VA Sierra Nevada Health Care System Medical Center in Reno and this is a follow up phone call to verify that an invitation letter to a research study was received.

Leave contact information of Study Coordinator: Rory Berk -- 775-328-1750

*****YOU CAN ONLY SPEAK TO THE PERSON IN QUESTION*****

III. When leaving voice mail for potential participants:

Hi, this is _____from the VA Sierra Nevada Health Care System Medical Center in Reno and this is a follow-up phone call to verify that you received the invitation letter to the research study titled **Pathogen and Biomarker Discovery in Gulf War Illness (GWI)** and to answer any questions you may have and to ask you if you would like researchers to find out if you are eligible. Please call……. at……..during……..hrs (Recruiter information). If for any reason you cannot reach me, please leave a message with a good time to reach you and I will call you as soon as I can. We appreciate your interest in the study. Thank You.

TITLE OF STUDY: "Pathogen and Biomarker Discovery in Gulf War Illness"
RESEARCHER(s): Vincent Lombardi, Ph.D. (775-682-8278); Elizabeth E. Hill, Ph.D., RN (775-328-1752); and Sheila Young, Ph.D. (775-786-7200 ext 1333)
PROTOCOL #: B12-036
SPONSOR: Department of Defense Gulf War Illness Research Program of the Office of the Congressionally Directed Medical Research Programs

Introduction

Before you agree to participate in this research study, it is important that you read and understand the following explanation of the study. It describes the purpose, procedures, benefits, risks, discomforts and precautions associated with the study. It describes your rights as a participant, including the right to withdraw from the study at any time. It is important to understand that no guarantee or assurances can be made regarding the results of the study. It is also important to understand that refusal to participate will not influence the standard treatment you receive. This consent may contain words that you do not understand. Please ask the investigator(s) to explain any words or information that you do not understand. It is essential that you be completely truthful regarding your health history and report any symptoms or reactions you may experience during the study. If you are not truthful, you may harm yourself by participating.

Purpose

You are being asked to participate in a research study done at the VA Sierra Nevada Health Care System (VASNHCS) and the Whittemore Peterson Institute (WPI). The purpose of this study is to investigate more about what causes Gulf War illness (GWI). Many people who were in the military during the Persian Gulf War (Desert Storm: 1990-1991) became ill and reported having symptoms that were common among them; these symptoms became known as GWI. However, we are still not sure what causes the illness. We hope that this study will help us to understand what causes it. In this study, we will be looking at differences in the immune system composition of subjects with GWI in comparison to subjects who don't have GWI. In order to do this, we will include veterans who served during the Persian Gulf War, whether or not they were deployed to Iraq or surrounding areas, and who have symptoms of GWI as well as subjects (veterans or non-veterans) who do not have symptoms of GWI.

Participants

We are asking you to participate in this research because you are a male or female, over the age of 18, fluent in English, and qualify under one of the following categories:

(a) You were on active duty in the military during the Persian Gulf War (Desert Storm: 1990-1991), you have symptoms often seen in what has been described as GWI, and you do not have or ever had a traumatic brain injury (TBI) or have human immunodeficiency virus (HIV). We would like for you to participate in this study even if you were not deployed to Iraq or surrounding areas during the Persian Gulf War, as long as you were on active duty during that time frame.

Or

(b) You have shown no symptoms of GWI and do not have a diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), traumatic brain injury (TBI), or human immunodeficiency virus (HIV).

As many as 200 people will participate in this study.

Procedures
If you are eligible to participate in the study, you will be asked to complete a participant questionnaire, which includes questions about your demographic characteristics (age, gender and location), your health and medical history, and, if applicable, information about your military service as it relates to the Persian Gulf War. You will also have about two tablespoons (30 ml) of blood drawn from a vein in your arm. We may ask you to provide additional samples only if needed for the research study. Your participation in this study will not take longer than 40 minutes and will be distributed more or less in the following manner:

- Complete participant questionnaire, which should take you no more than 5-10 minutes.
- Provide a blood sample. It is possible you might have to wait a few minutes to have your blood drawn, but that should take no more than 20-30 minutes total.

Alternatives
You may choose not to participate in this study.

Discomforts, Inconveniences, and/or Risks
You will experience brief pain from the needle stick for the blood draw. Less than two tablespoons (30ml) of blood will be drawn. Sometimes people have some bruising or swelling at the site where the blood is drawn. There is a possible risk of infection at the site where the blood is drawn, but that is extremely rare. On very rare occasions the person having blood drawn faints, but that seldom happens.

Sometimes people feel uncomfortable when they are asked questions about their health. If you are feeling uncomfortable you may refuse to answer any questions. You can also say that you do not want to answer certain questions on the participant questionnaire or that you wish to stop participating in the research.

Benefits
There may be no direct benefits to you as a participant in this study. However, there are possible benefits to science from the knowledge gained in this study. This research will help us to better understand GWI and could lead to the development of better treatments or prevention.

TITLE OF STUDY: "Pathogen and Biomarker Discovery in Gulf War Illness"
RESEARCHER(s): Vincent Lombardi, Ph.D. (775-682-8278); Elizabeth E. Hill, Ph.D., RN (775-328-1752); and Sheila Young, Ph.D. (775-786-7200 ext 1333)
PROTOCOL #: B12-036
SPONSOR: Department of Defense Gulf War Illness Research Program of the Office of the Congressionally Directed Medical Research Programs

Confidentiality
The researchers, the VA Sierra Nevada Health Care System, the WPI, and the Department of Defense will treat your identity with professional standards of confidentiality and protect it to the extent allowed by law. However, there are reasons why people other than the researchers may need to see information you provided as part of the study. This includes organizations responsible for making sure the research is done safely and properly, including government research offices such as The Department of Health and Human Service; the Department of Veterans Affairs; the University of Nevada, Reno Biomedical Institutional Review Board; and the study sponsor, Department of Defense Gulf War Illness Research Program of the Office of the Congressionally Directed Medical Research Programs. Also, if you tell us something that makes us believe that you or others have been or may be physically harmed, we may report that information to the appropriate agencies.

You will be assigned a random number, and only designated researchers will have access to the list which matches that random number to your name. The list, the informed consent documents, and the participant questionnaires will not be provided to the WPI and will be maintained in locked file cabinets behind locked doors at the VA Research Offices. However, data collected from the participant questionnaires will be shared with the WPI. This data will be sent coded (un-identified) and it will be stored in password protected computers at the WPI. Only your name, address, and social security number will be given to Sierra Veterans Research and Education Foundation so that your study time/travel stipend can be processed. Blood samples will be labeled with the code assigned to you and sent to the WPI where they will be kept securely locked in freezers in the research laboratory. Labels on blood samples will not include your name, only your code number. All WPI offices, labs, and files are locked when not occupied. We plan to publish the results of this research, but will not include any information that would identify you.

We will enroll participants into this research for approximately two years and study data and blood samples sent to WPI will be retained there for two years after the study is completed. At that time, all data and blood samples kept at WPI will be destroyed. In addition, materials kept at the VA will also be destroyed in accordance with the VA Record Control Schedule.

Costs/Compensation
There will be no cost to you for participating in this research study. However, we would like to compensate you for your time and travel associated with participation in the study. You will receive a $25 check sent from the Sierra Veterans Research and Education Foundation. If this research leads to the development of commercial products (like a test to detect a disease) or discoveries that could be patented, registered, or otherwise developed for commercial sale, you will not receive any financial benefit from

that. You will not have patent or ownership rights to any products or discoveries resulting from this research.

The Department of Veterans Affairs will provide necessary medical treatment if you are injured by participation in a research project approved by a VA R&D Committee and conducted under the supervision of one or more VA employees. Except in limited circumstances, the necessary care will be provided in VA medical facilities. Exceptions include: situations where VA facilities are not capable of furnishing economical care; situations where VA facilities are not capable of furnishing the care or services required; and some situations involving a non-veteran research subject. This requirement does not apply to treatment for injuries that result if you do not comply with study procedures.

Some veterans are required to pay co-payments for medical care and services provided by VA. These co-payment requirements will continue to apply to medical care and services provided by VA that are not part of this study. For further information, please contact the Research Compliance Officer at 775-786-7200, Ext. 1177.

Disclosure of Financial Interests
This research is being funded by the Department of Defense Gulf War Illness Program Office of the Congressionally Directed Medical Research Programs. This grant will support in part salaries of research personnel at the WPI and will pay for a study coordinator to help manage the research. There is no individual financial gain for any of the researchers involved in this research.

Right to Refuse or Withdraw
You may refuse to participate or withdraw from the study at any time. Choosing not to participate will not affect the care you receive from your regular health care provider(s). If something changes in the study, we will tell you about it and get your consent to continue participation if you choose to do so.
You will be told about any important new information that may change your mind about staying in the study.

Permission to be Contacted for Future Studies

TITLE OF STUDY: "Pathogen and Biomarker Discovery in Gulf War Illness"
RESEARCHER(s): Vincent Lombardi, Ph.D. (775-682-8278); Elizabeth E. Hill, Ph.D., RN (775-328-1752); and Sheila Young, Ph.D. (775-786-7200 ext 1333)
PROTOCOL #: B12-036
SPONSOR: Department of Defense Gulf War Illness Research Program of the Office of the Congressionally Directed Medical Research Programs

You can decide if you want to be contacted to participate in future research studies. In this case, you are being asked whether WPI researchers have permission to contact you in the future if you are eligible to participate in a future research study. New blood samples would be collected, provided you were eligible and consented to be part of any such future research study. Your decision can be changed at any time by notifying the principal investigator or the study coordinator in writing. Your decision about your future participation will not affect your participation in this study or any other studies or the medical care you receive from your regular health care professional(s).

Please initial your decision about permission to be contacted for future research studies:

_____ YES, you may contact me to participate in future research studies.

_____ NO, you may not contact me to participate in future research studies.

Questions
If you have questions about this study or wish to report a research-related problem, please contact Vincent Lombardi, Ph.D. (775-682-8278); Elizabeth E. Hill, Ph.D., RN (775-328-1752); or Rory Berk (775-328-1750) at any time.

You may ask about your rights as a research subject. If you have questions, concerns or complaints, you may report them (anonymously if you so choose) to the VA Sierra Nevada Health Care System's Research Compliance Officer, telephone number 775-786-7200 x1177, or to the University of Nevada, Reno Biomedical Institutional Review Board, telephone number (775) 327-2368, or by addressing a letter to the Chair of the Board, c/o UNR Office of Human Research Protection, 205 Ross Hall / 331, University of Nevada, Reno, Reno, Nevada, 89557.

TITLE OF STUDY: "Pathogen and Biomarker Discovery in Gulf War Illness"
RESEARCHER(s): Vincent Lombardi, Ph.D. (775-682-8278); Elizabeth E. Hill, Ph.D., RN (775-328-1752); and Sheila Young, Ph.D. (775-786-7200 ext 1333)
PROTOCOL #: B12-036
SPONSOR: Department of Defense Gulf War Illness Research Program of the Office of the Congressionally Directed Medical Research Programs

Closing Statement

I have read ( ) this consent form or have had it read to me ( ).

_____has explained the study to me and all of my questions have been answered. I have been told of the risks or discomforts and possible benefits of the study.

If I do not take part in this study, my refusal to participate will involve no penalty or loss of rights to which I am entitled. I may withdraw from this study at any time without penalty or loss of VA medical care/benefits or other benefits to which I am entitled.

I have been told my rights as a research subject, and I voluntarily consent to participate in this study. I have been told what the study is about and how and why it is being done. All of my questions have been answered.

I will receive a signed and dated copy of this consent form.

Signature of Participant Date

Printed Name of Participant

Signature of Person Obtaining Consent Date

Signature of Witness (if applicable) Date

University of Nevada, Reno

Office of Human Research Protection
218 Ross Hall / 331, Reno, Nevada 89557
775.327.2368 / 775.327.2369 fax
www.unr.edu/ohrp

II2 FO5 Protocol Modification Request

Date: 08/20/12

Principal Investigator: Vincent C. Lombardi, PhD.
Protocol Title: Pathogen and Biomarker Discovery in Gulf War Illness
Protocol Number: B12-036

1. Proposed Protocol Modifications
   Required: Two copies of the revised and updated protocol application
   As applicable: Two copies of the revised and updated supporting documents
   1.a. List the modifications being requested: Flyer v3, Invitation Letter v3, Telephone Script
   1.b. Justify or state the reason for the modifications: Flyer and Invitation Letter: Minor clarification/ See document for marked changes . Telephone Script: Minor clarification and change of phone number for Study Coordinator. See marked copy.

2. Change in Principal Investigator
   Required: Two copies of the revised and updated protocol application, *signed by the current PI*.
   Name and title of new PI:

3. Changes in Research Personnel
   Required: Two copies of the revised and updated protocol application
   ☐ Removing co-investigators / research personnel
   Provide names of researchers being removed:
   ☐ Adding co-investigators / research personnel
   ☐ All new researchers have completed the required human subjects training
   Provide names and titles of researchers being added:

   3.a. Do any of the researchers being added to the protocol at this time have a conflict of interest, either financial or non-financial?
   ☐ No
   ☐ Yes, complete table 3b below.

Table 3b Researcher Conflict of Interest Information

Name	Has a SFI Disclosure Form been submitted to the Office of Sponsored Projects?	Does the Office of Human Research Protection have a copy of the Management Plan?
	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No
	☐ Yes ☐ No	☐ Yes ☐ No

4. Changes in Sponsorship
   Required: Two copies of the revised and updated protocol application
   Required: For each new sponsor, two copies of the grant proposal or contract with scope of work
   As applicable: Two copies of the sponsor protocol
   ☐ Removing sponsors
   List sponsors being removed:
   ☐ Adding sponsors
   List sponsors being added:

5. Do any of the modifications require changes to the consent documents?
   ☒ No
   ☐ Yes, attach two copies of the revised and updated consent documents and summarize the amendments:

6. Principal Investigator Assurance
   I hereby certify that all information provided with this request is complete and accurate.

   For investigator/personnel changes: I hereby certify that all responsible investigators added above are appropriately credentialed and/or trained to perform their role in this protocol. I further certify that the participation of any co-investigators or research personnel listed above does not, in any way, violate the University of Nevada, Reno policy on conflicts of interest.

Signature of Principal Investigator* Date

*Current PI must sign unless the current PI is unavailable and the Responsible Official may sign.

University of Nevada, Reno

Office of Human Research Protection
218 Ross Hall / 331, Reno, Nevada 89557
775.327.2368 / 775.327.2369 fax
www.unr.edu/ohrp

Certification of Approval for Modifications
Biomedical Institutional Review Board
FWA00002306

Date: September 25, 2012
To: Vincent C Lombardi, PhD Department of Pathology and Laboratory Medicine
Copy: Research Office VASNHCS
Craig Ballard

UNR Protocol Number:	B12-036
Protocol Title:	Pathogen and Biomarker Discovery in Gulf War Illness
Sponsor Names:	US Department of Defense
Type of Review:	Expedited 2, 5 & 7 Minimal risk
Meeting/Review Date:	September 25, 2012
Approval Period:	June 26, 2012 to June 25, 2013

This approval is for:
Approved number of subjects: 200
Approved documents: Flier (INV Recruitment Materials)
Addition of new flier for veteran population recruitment

The above-referenced protocol was reviewed and approved by one of UNR's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56).

Problems Researchers Must Report to the Research Integrity Office or IRB Staff
(to be reported as soon as possible, but within 10 business days)

- New or additional risks: Outcomes that the principal investigator believes are unexpected, related to the research, and suggest the research may place participants or others at greater risk of harm than was previously known or recognized
- Changes to expected harms or benefits: Any report indicating the frequency or magnitude of harms or benefits may be different than initially presented to the IRB
- Privacy: Any invasion of privacy related to an individual's participation in research
- Confidentiality: Any breach of confidentiality involving research data
- FDA Changes: Any change in FDA labeling or approval for a drug, device or biologic used in a research protocol
- Immediate harm: Any change to the protocol to eliminate an apparent immediate hazard to a research participant, prior to seeking IRB review and approval
- Prisoner: Any incarceration of a participant in a protocol not approved to enroll prisoners
- Sponsor: Any event that requires prompt reporting to the sponsor
- Sponsor: Any sponsor-imposed suspension for risk
- Protocol change: Any accidental or unintentional change to the IRB approved protocol that harmed participants or others, indicates participants or others may be at increased risk of harm, or has the potential to recur
- Device: Any unanticipated adverse device effect

- Department of Health: Any non-compliance identified by Department of Health audit or monitoring
- Federal agency: Any investigation or report by federal agency related to the research
- Medical license or practice changes: Any loss of license or hospital privileges by any researcher on the study
- Complaints: Any complaints that suggest participants or others may have been harmed or placed at increased risk of harm

PI Responsibilities

- Maintain an accurate and complete protocol file.
- Submit continuing projects for review and approval prior to the expiration date.
- Submit proposed changes for review and approval prior to initiation, except when necessary to eliminate apparent immediate hazards to subjects. Such exceptions must be reported to the IRB at once.
- Report any unanticipated problems which may increase risks to human subjects or unanticipated adverse events to the IRB within 5 days.
- Submit a closure request 10 days after project completion to the IRB.

Reference the protocol number on all related correspondence with the IRB. If you have any questions, please contact Valerie Smith at 775.327.2368.

For Veteran's Administration research only

VA Research: Yes
Flag VA Medical Record: No

DEPARTMENT OF VETERAN AFFAIRS
VA SIERRA NEVADA HEALTH CARE SYSTEM

PARTICIPANT QUESTIONNAIRE

INTERNAL USE ONLY

Participant Number:_____

PERSONAL INFORMATION

Last Name: _____ First Name: _____ MI: _____

☐ Male ☐ Female Date of Birth: _____

Street Address: _____ City: _____

State: _____ Zip: _____ Country: _____

Home Phone: _____

Work Phone: _____

Cell Phone: _____

Email: _____

All information is personal

INTERNAL USE ONLY

Participant Number:_____

MEDICAL HISTORY

Primary Diagnosis

Myalgic Encephalomyelitis (ME)/Chronic Fatigue Syndrome (CFS)	☐Yes	☐No
Fibromyalgia	☐Yes	☐No
Gulf War illness	☐Yes	☐No
Date of Onset: _____		
Date of Diagnosis: _____		

Initial Symptoms

Headache	☐Yes	☐No
Sore throat	☐Yes	☐No
Painful muscles	☐Yes	☐No
Fever	☐Yes	☐No
Rash	☐Yes	☐No
Painful joints	☐Yes	☐No
Gastrointestinal disorders	☐Yes	☐No
Nerve pain	☐Yes	☐No
Disturbed balance	☐Yes	☐No
Profound weakness	☐Yes	☐No
Difficulty with short term memory	☐Yes	☐No
Difficulty with mental processing	☐Yes	☐No
Flu-like illness	☐Yes	☐No
Gradual onset	☐Yes	☐No
Onset associated with chemical exposure	☐Yes	☐No

INTERNAL USE ONLY

Participant Number:_____

Current Medications

Non-steroid anti-inflammatory	☐Yes	☐No
Steroid anti-inflammatory	☐Yes	☐No
Antivirals	☐Yes	☐No
Antibiotics	☐Yes	☐No

Other prescription drugs *(please list)* _____

_____

_____

_____

Co-Infections

Epstein Barr virus (EBV)	☐Yes	☐No
Cytomegalovirus (CMV)	☐Yes	☐No
Lyme disease	☐Yes	☐No
HIV	☐Yes	☐No
HTLV	☐Yes	☐No
Parvo virus	☐Yes	☐No
Enterovirus	☐Yes	☐No
Coxsackievirus	☐Yes	☐No
V2 Virus (Chicken Pox)	☐Yes	☐No

Secondary Diagnosis			***Date of Diagnosis***
Multiple sclerosis	☐Yes	☐No	_____
Lupus	☐Yes	☐No	_____
Encephalopathy	☐Yes	☐No	_____
Psoriasis	☐Yes	☐No	_____
Asthma	☐Yes	☐No	_____
Cardiovascular disease	☐Yes	☐No	_____
Crohn's disease	☐Yes	☐No	_____
Diabetes	☐Yes	☐No	_____
Cancer Type_____	☐Yes	☐No	_____
Pulmonary disease	☐Yes	☐No	_____

INTERNAL USE ONLY

Participant Number:_____

Secondary Diagnosis (Continued)

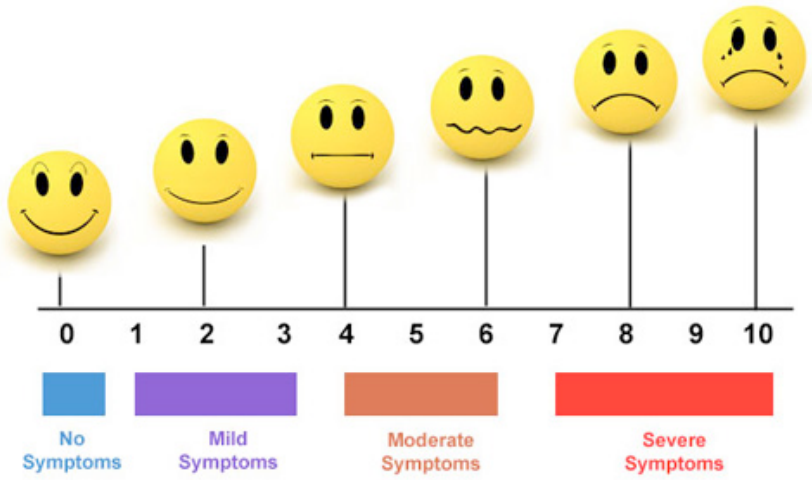
			Date of Diagnosis
Neurological disease	☐Yes	☐No	_____
Rheumatoid arthritis	☐Yes	☐No	_____
Sjogren's syndrome	☐Yes	☐No	_____
Cognitive disorders	☐Yes	☐No	_____
Post traumatic stress syndrome	☐Yes	☐No	_____
Traumatic brain injury	☐Yes	☐No	_____
Depression	☐Yes	☐No	_____

Miscellaneous

Have you ever had any surgeries?	☐Yes	☐No
Do you have any metal implants (including dental implants, titanium bone pins, etc.?)	☐Yes	☐No
Do you have any non-metal implants?	☐Yes	☐No
Have you ever had a blood transfusion?	☐Yes	☐No
Have you ever had an adverse reaction to a vaccination?	☐Yes	☐No
Have you ever had any experimental vaccines?	☐Yes	☐No

Chronic Pain

Using the scale to the right, please circle the number which best describes your current level of chronic pain.



INTERNAL USE ONLY

Participant Number:_____

Exercise/Activity Limitations

Using the scale below, please indicate how your health limits you while performing the following activities.

1	Not limited at all
2	Somewhat limited
3	Extremely limited
4	Intolerable

Vigorous activities (i.e. running, lifting heavy objects, participating in strenuous sports)	☐ 1	☐ 2	☐ 3	☐ 4
Moderate activities (i.e. moving a table, pushing a vacuum cleaner, bowling, or playing golf)	☐ 1	☐ 2	☐ 3	☐ 4
Lifting or carrying groceries	☐ 1	☐ 2	☐ 3	☐ 4
Climbing several flights of stairs	☐ 1	☐ 2	☐ 3	☐ 4
Climbing one flight of stairs	☐ 1	☐ 2	☐ 3	☐ 4
Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3	☐ 4
Walking more than a mile	☐ 1	☐ 2	☐ 3	☐ 4
Walking one block	☐ 1	☐ 2	☐ 3	☐ 4
Bathing or dressing yourself	☐ 1	☐ 2	☐ 3	☐ 4

MILITARY SERVICE (IF VETERAN RESEARCH SUBJECT)

Did you serve in the Persia n Gulf War (Desert Storm 1990-1991)? ☐Yes ☐No

Did your service include deployment to the "Gulf Theater of Operations"? (Includes Iraq, Kuwait, Saudi Arabia, Bahrain, Qatar, the United Arab Emirates, Oman, the Gulf of Aden, the Gulf of Oman, the Persian Gulf, the Arabian Sea, the Red Sea, and the airspace above all of these locations). ☐Yes ☐No

If deployed:

Where deployed?

_____

Dates of deployment?

_____

The VA Sierra Nevada Health Care System and Whittemore Peterson Institute are conducting a research Study:

Pathogen and Biomarker Discovery in Gulf War Illness

Attention All • Volunteers Needed

The purpose of this study is to investigate the potential causes of GWI. Researchers will be looking at differences in the immune system composition between veterans that have symptoms of GWI and subjects that do not have those symptoms, which in turn will afford physicians the necessary tools to make more accurate diagnoses.

Who is eligible?

- Veterans who were on active duty during the Gulf War (1990 - 1991) and who have been seen in the VA Health Care System.

What will you be asked to do?

- Complete a brief questionnaire (5-10minutes)
- Provide 30ml (about 2 tablespoons) of blood (20-30 minutes)

Costs and Compensation

- You will receive $25 compensation for travel and time associated with study participation

This study will be performed at the Ioannis A. Lougaris VA Medical Center in Reno and the VA Sierra Nevada Health Care System Community Based Outpatient Clinics.*

For more information or to volunteer for this study, please contact:
Study Coordinator Rory Berk ~ 775-328-1750

****Processing and analysis of blood samples will be conducted at the Whittemore Peterson Institute (1664 N Virginia St., University of Nevada, Reno) under the supervision of the Principal Investigator, Dr. Vincent Lombardi (775-682-8278)***

Rory Berk (775) 328-1750
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Who is eligible?

- No symptoms that have been associated with GWI Syndrome
- Male or female
- 18 years old or older
- Literate in English
- No diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) or similar neuro-immune disease, traumatic brain injury (TBI), or human immunodeficiency virus (HIV)

What will you be asked to do?

- Complete a brief questionnaire (5-10minutes)
- Provide 30ml (about 2 tablespoons) of blood (20-30 minutes)

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